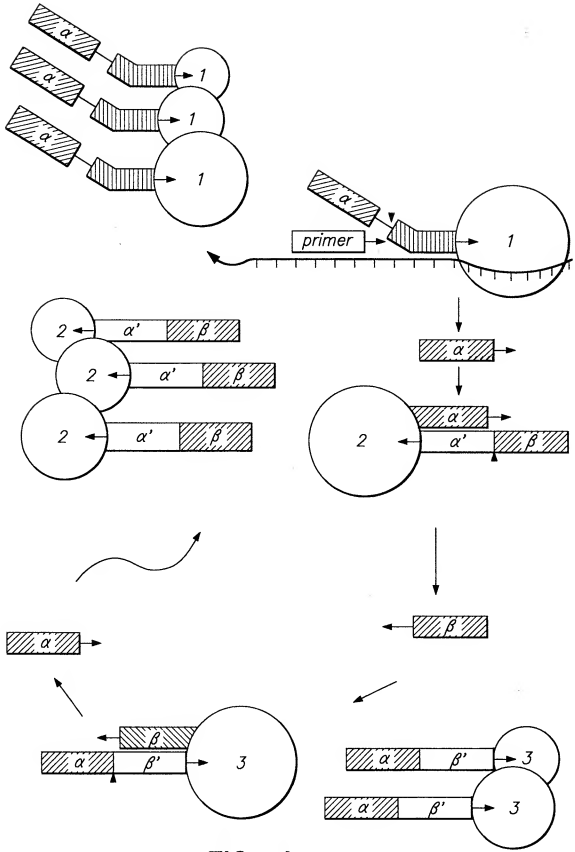
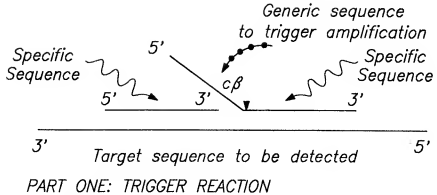


FIG. 1A

FIG. 1B


Generic sequence
to trigger amplification
Specific
Sequence
5'
cβ
Specific
Sequence
5'
3'
3'
3'
5'
Target sequence to be detected
PART ONE: TRIGGER REACTION

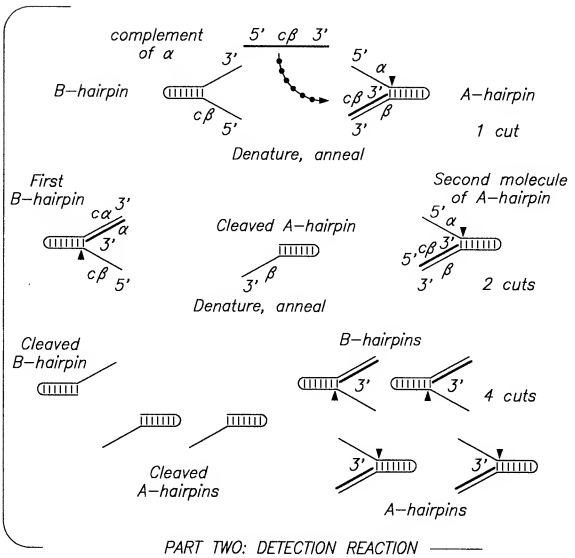

complement
of α
5' cβ 3'
3'
5'
α
B–hairpin
cβ
3'
A–hairpin
cβ
5'
β
3'
1 cut
Denature, anneal
First
B–hairpin
3'
cα
α
3'
cβ
5'
Cleaved A–hairpin
3'
β
Second molecule
of A–hairpin
5'
α
5'
cβ
3'
3'
β
2 cuts
Denature, anneal
Cleaved
B–hairpin
B–hairpins
3'
3'
4 cuts
Cleaved
A–hairpins
3'
3'
A–hairpins
PART TWO: DETECTION REACTION

FIG. 2A

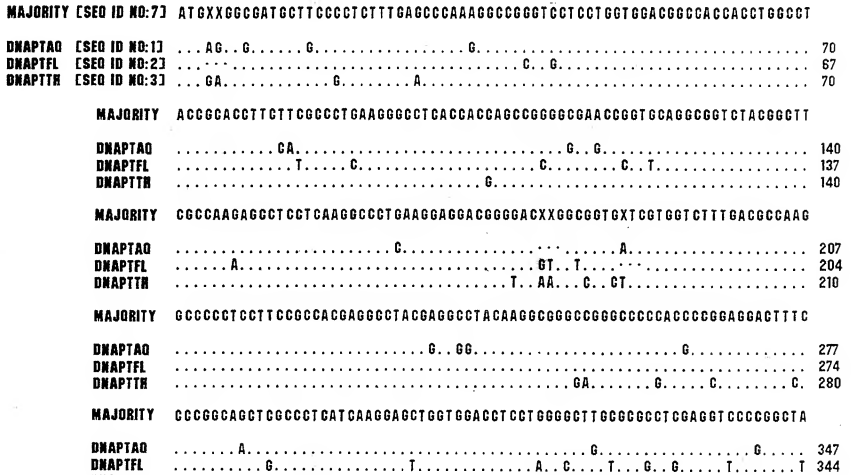


FIG. 2B

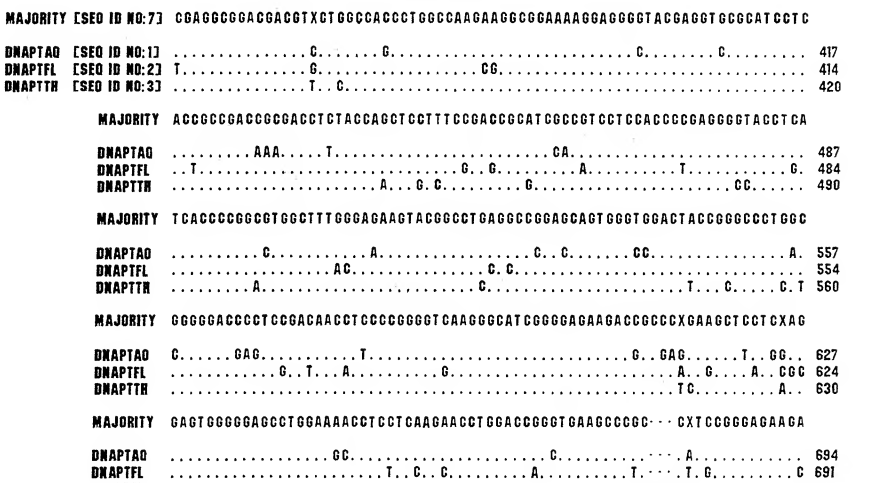


FIG. 2C

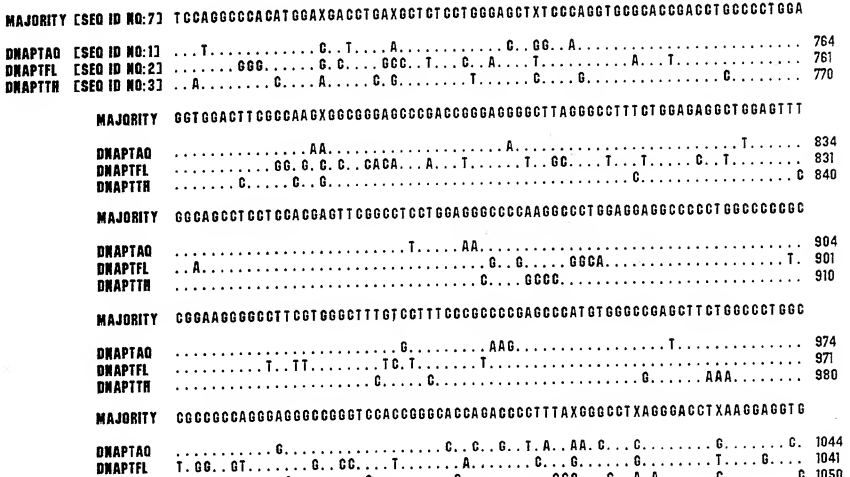


FIG. 2D

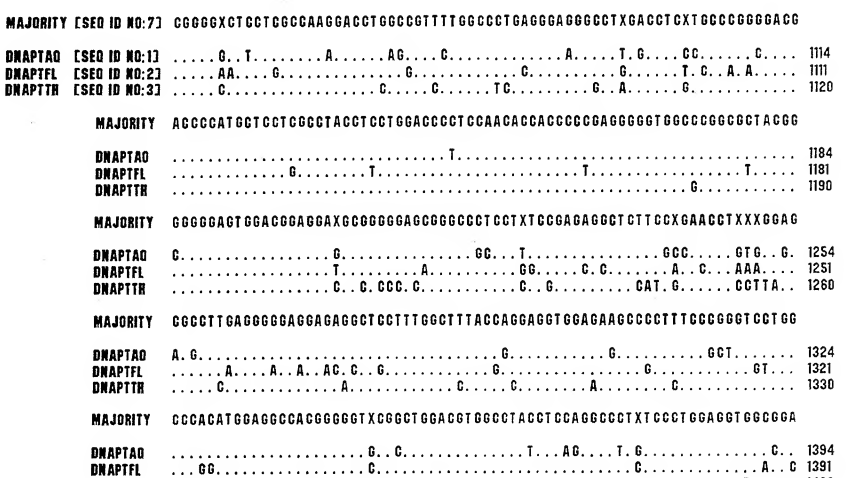


FIG. 2E

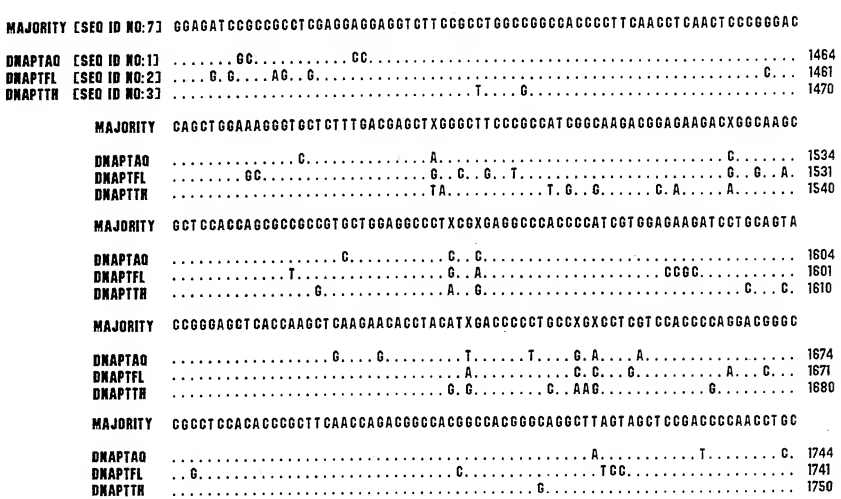


FIG. 2F

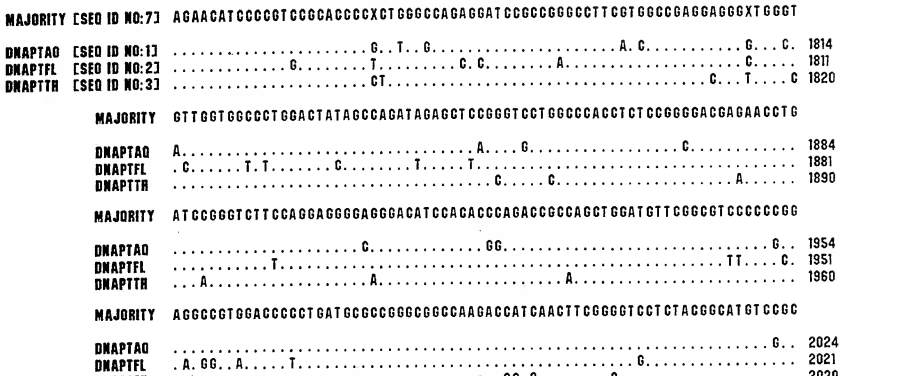


FIG. 2G

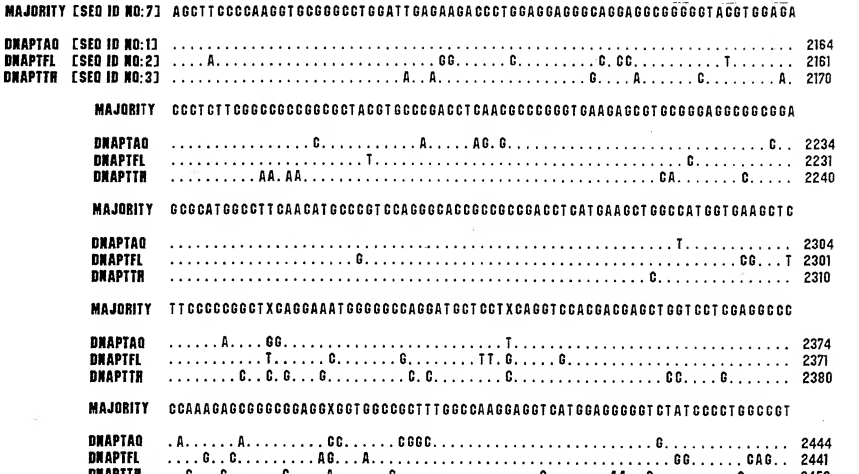


FIG. 2H

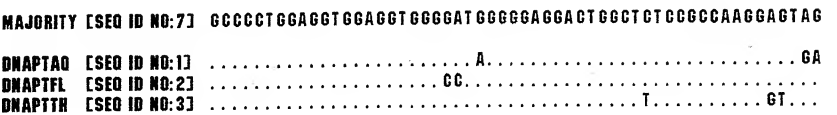


FIG. 3A

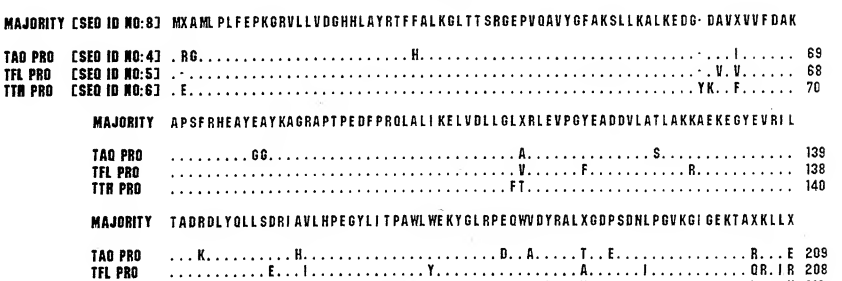
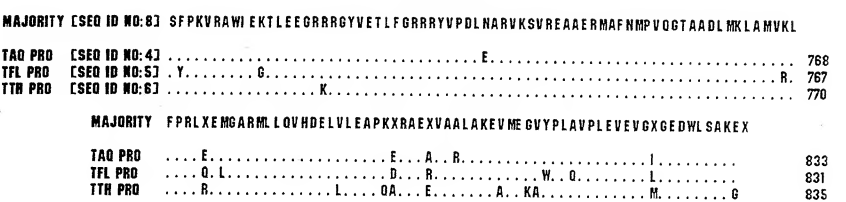


FIG. 3B

FIG. 3C



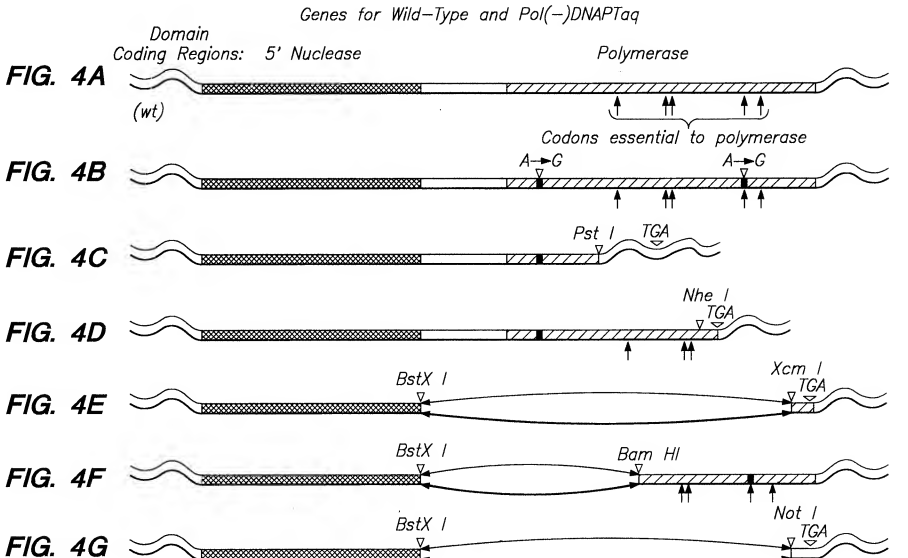


FIG. 4A

FIG. 4B

FIG. 4C

FIG. 4D

FIG. 4E

FIG. 4F

FIG. 4G

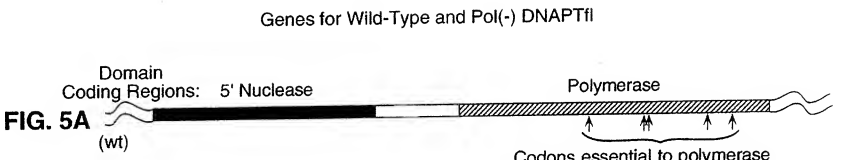


FIG. 5A

FIG. 5B

5' arm
TAATACGACTCACTATAGGGAGACC
(SEQ ID NO:164)
Cleavage Sites
a
b
Substrate Strand
CCGGAATTCGAGCTCGC C
GCCTTAAGCTCGAGCG G
Template Strand
3'
5'
GAATT CGATTTAGGTGACACTATAGAA
primer
CTTAAGCTAAATCCACTGTGATATCTTATGT
3 arm
(SEQ ID NO:17)

FIG. 6

FIG. 7

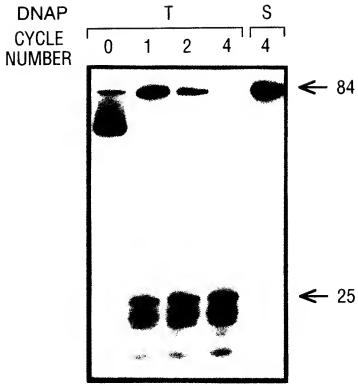


FIG. 8

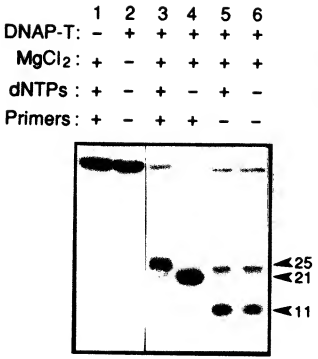


FIG. 9A

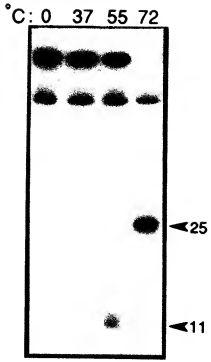


FIG. 9B

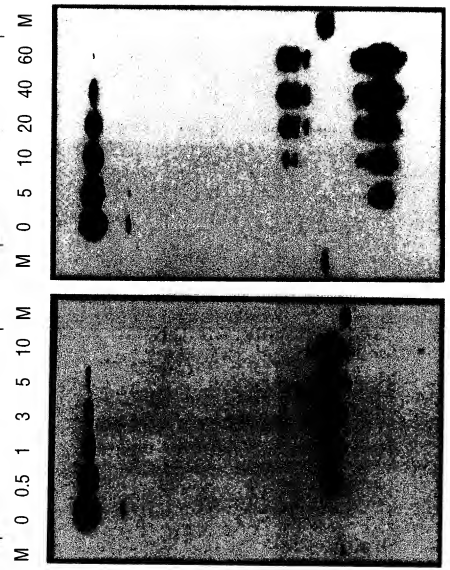


FIG. 10A

FIG. 10B

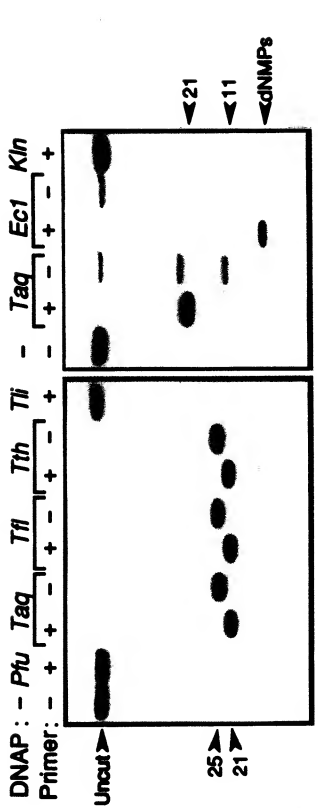
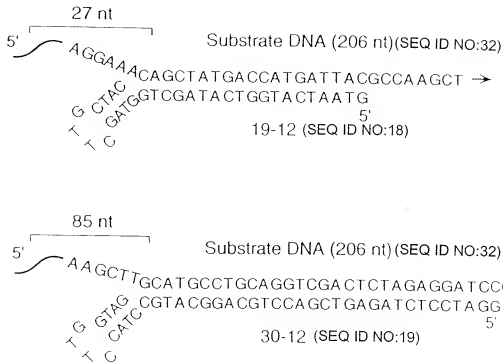


FIG. 11A

FIG. 11B

FIG. 12A


27 nt
5'
AGGAAACAGCTATGACCATGATTACGCCAAGCT →
GTCGATACTGGTACTAATG
5'
19-12 (SEQ ID NO:18)
Substrate DNA (206 nt)(SEQ ID NO:32)
85 nt
5'
AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCC →
CGTACGGACGTCCAGCTGAGATCTCCTAGG
5'
30-12 (SEQ ID NO:19)
Substrate DNA (206 nt) (SEQ ID NO:32)

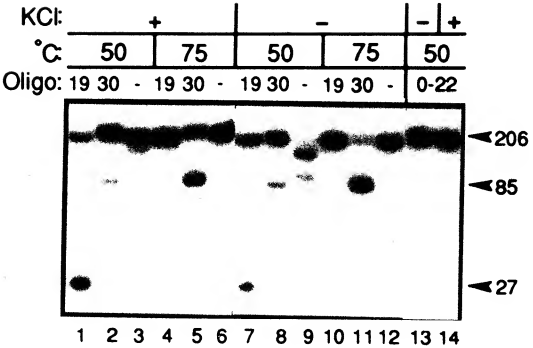


FIG. 12B

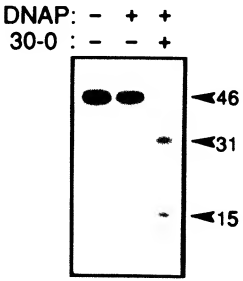


FIG. 13B

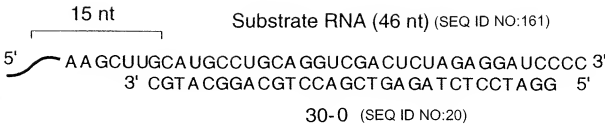

15 nt
Substrate RNA (46 nt) (SEQ ID NO:161)
5'
A A GCUUGCA UGCCUGCA GGUCGA CUCUA GA GGA UCCCC 3'
3' CGT A CGGA CGT CCA GCT GA GA T CT CCT A GG 5'
30-0 (SEQ ID NO:20)

FIG. 13A

(SEQ ID NO:162)

-35 -10 RBS

TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG

MetAsnSer...

ATGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCC

EcoRI KpnI BamHI SalI SphI

SstI SmaI XbaI PstI HindIII

FIG. 14B

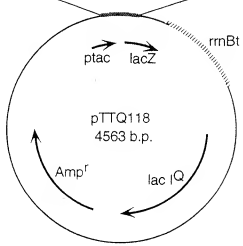


FIG. 14A

RBS: Ribosome binding site
ptac: Synthetic tac promoter
lac I^Q: Lac repressor gene
lacZ: Beta-galactosidase alpha fragment
rrnBt: E. coli rrnB transcription terminator

FIG. 14C

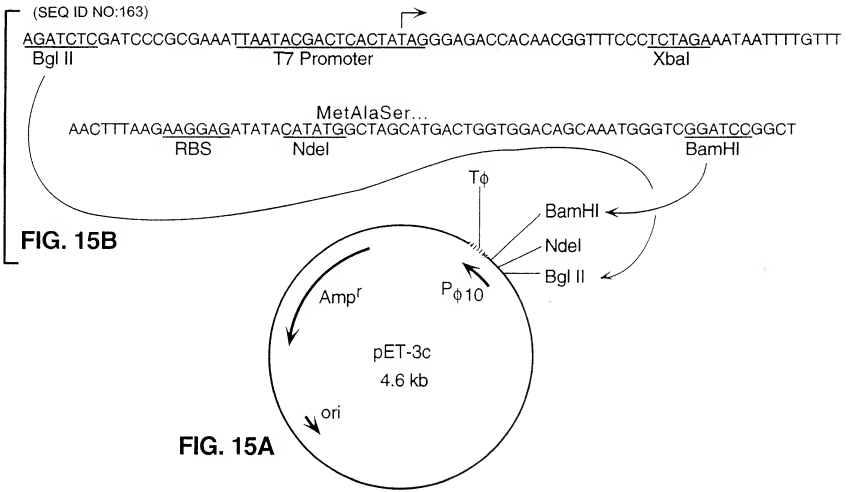


FIG. 15B

FIG. 15A

$P_{\phi 10}$: Bacteriophage T7 ϕ10 promoter RBS: Ribosome binding site
Tϕ: T7 ϕ Terminator

FIG. 15C

FIG. 16A

FIG. 16B

FIG. 16C

FIG. 16D

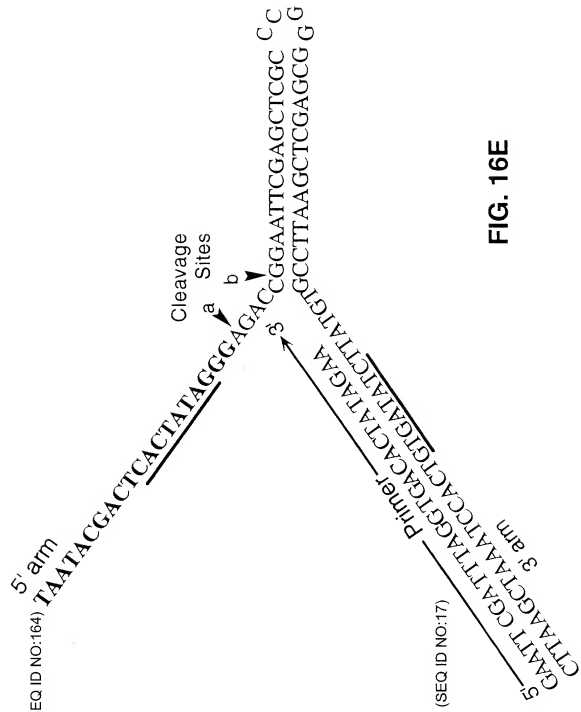

5′ arm
(SEQ ID NO:164) TAATACGACTCACTATAGGGAGACCGGAATTCGAGCTCGC C C
GCCTTAAGCTCGAGCG G G
Cleavage Sites
a
b
3′
Primer
(SEQ ID NO:17)
5′ GAATT CGATTTAGGTGACACTATAGAA
CTTAAGCTAAATCCACTGTGATATCTTAT GTT
3 arm

FIG. 16E

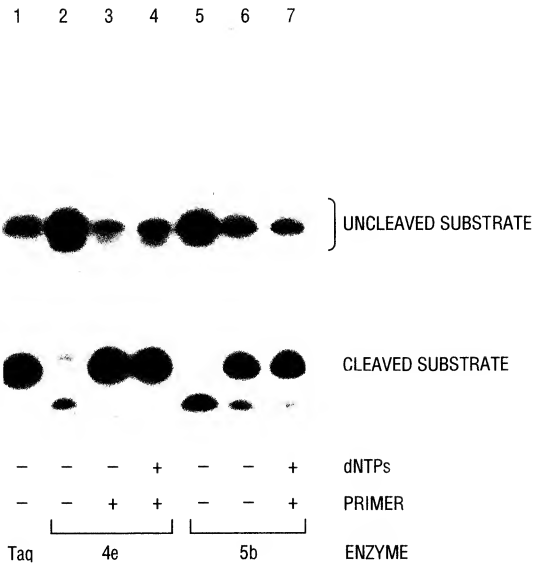


FIG. 17

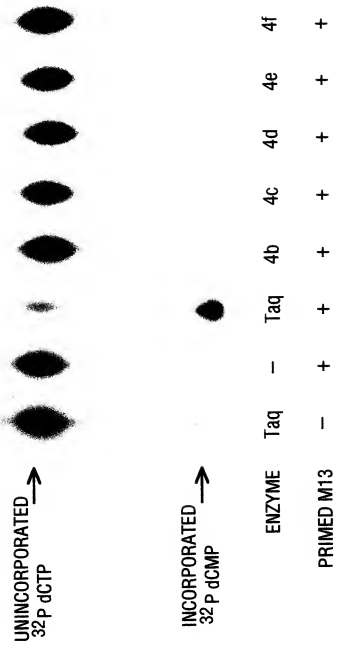

UNINCORPORATED
32P dCTP
INCORPORATED
32P dCMP
ENZYME Taq – Taq 4b 4c 4d 4e 4f
PRIMED M13 – + + + + + + +

FIG. 18

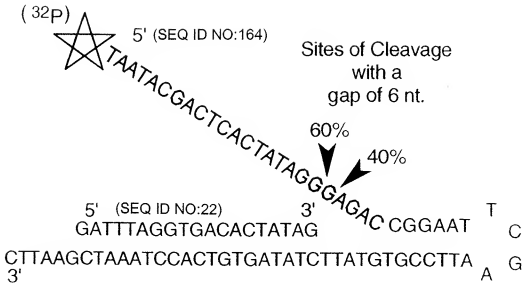

(32P)
5' (SEQ ID NO:164)
TAATACGACTCACTATAGGGAGAC
Sites of Cleavage
with a
gap of 6 nt.
60%
40%
5' (SEQ ID NO:22)
GATTTAGGTGACACTATAG
3'
CGGAAT
T
C
G
A
CTTAAGCTAAATCCACTGTGATATCTTATGTGCCTTA
3'

FIG. 19A

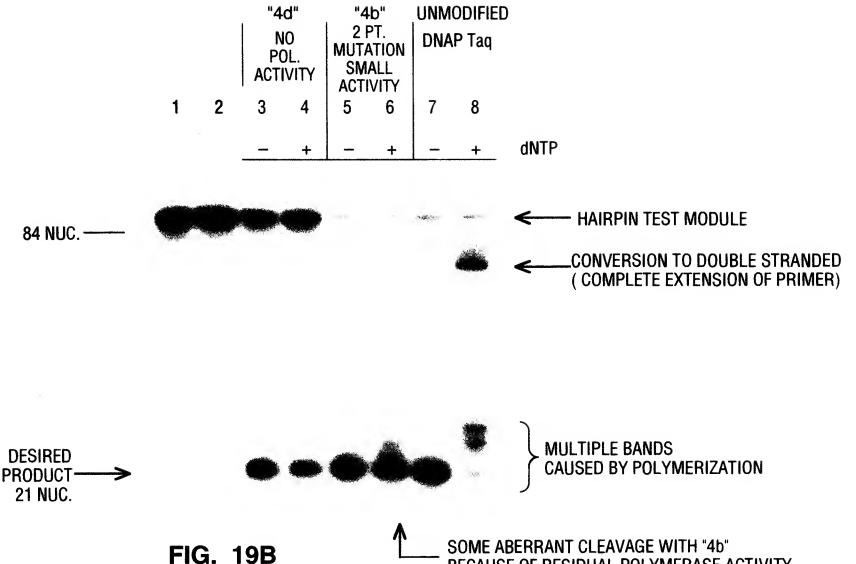


FIG. 19B

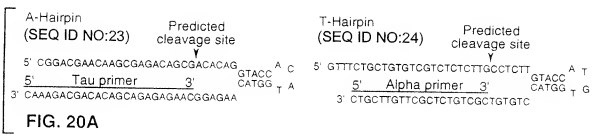


FIG. 20A

Sequence of alpha primer: (SEQ ID NO:25)

5' GACGAACAAGCGAGACAGCG 3'

FIG. 20B

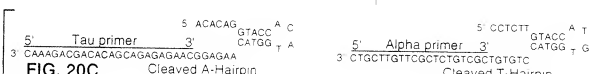


FIG. 20C

Cleaved A-Hairpin
(SEQ ID NO:28)

Cleaved T-Hairpin
(SEQ ID NO:27)

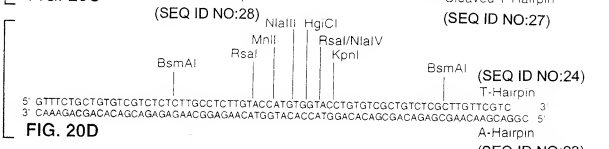


FIG. 20D

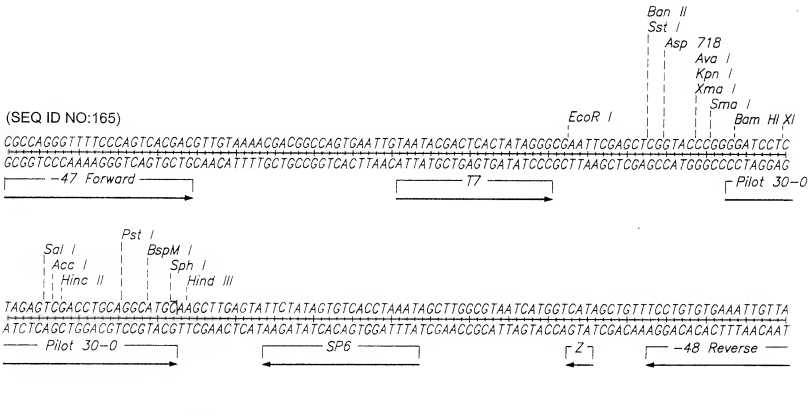


FIG. 21

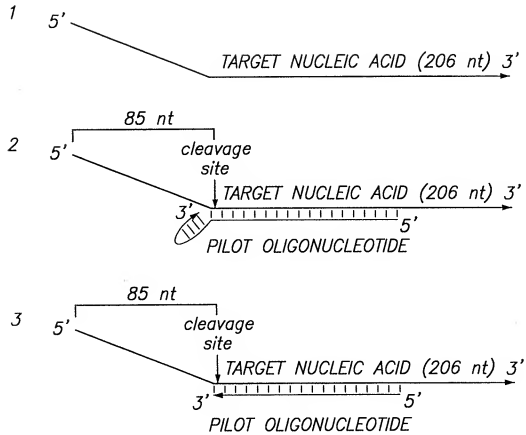

1
5'
TARGET NUCLEIC ACID (206 nt) 3'
85 nt
2
5'
cleavage
site
TARGET NUCLEIC ACID (206 nt) 3'
3'
5'
PILOT OLIGONUCLEOTIDE
85 nt
3
5'
cleavage
site
TARGET NUCLEIC ACID (206 nt) 3'
3'
5'
PILOT OLIGONUCLEOTIDE

FIG. 22A

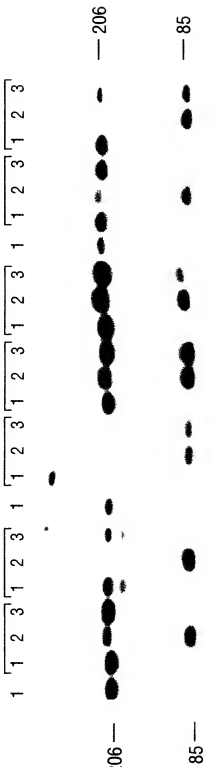


FIG. 22B

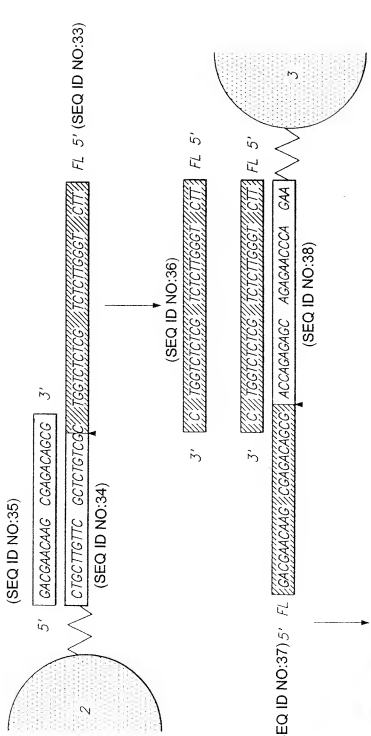

(SEQ ID NO:35)
5'
GACGAACAAG CGAGACAGCG
3'
2
CTGCTTGTTC GCTCTGTCGC
(SEQ ID NO:34)
FL 5' (SEQ ID NO:33)
(SEQ ID NO:36)
3'
FL 5'
3'
FL 5'
(SEQ ID NO:37) 5'
FL
ACCAGAGAGC AGAGAACCCA GAA
(SEQ ID NO:38)
3
5'
FL
(SEQ ID NO:35)
3'

FIG. 23

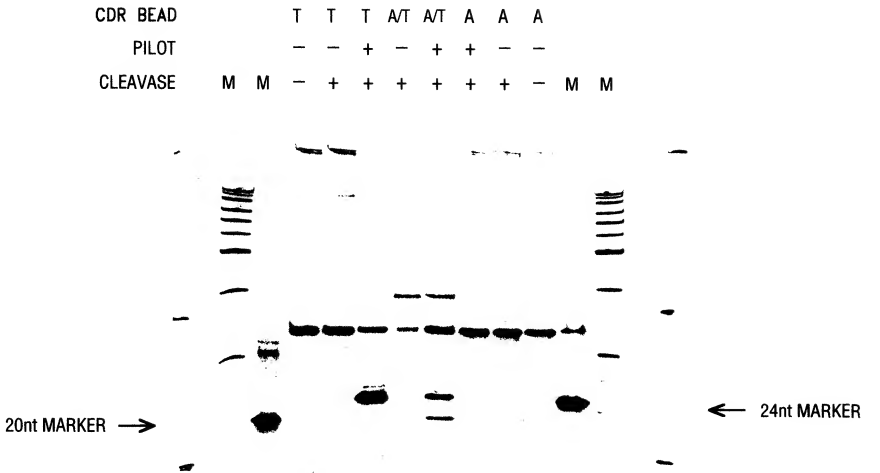


FIG. 24

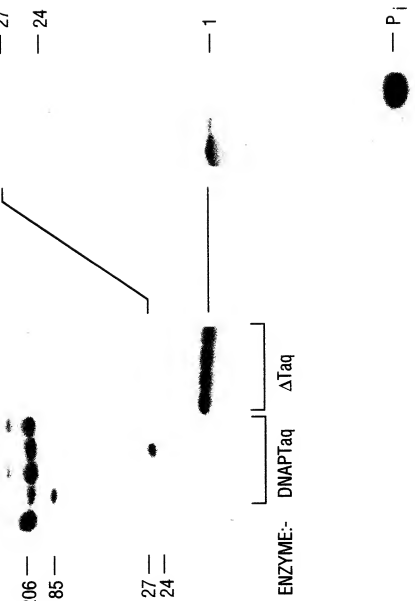
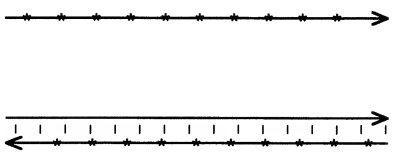


FIG. 25A

FIG. 25B

FIG. 26A



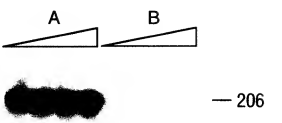


FIG. 26B

* = ^{32}P

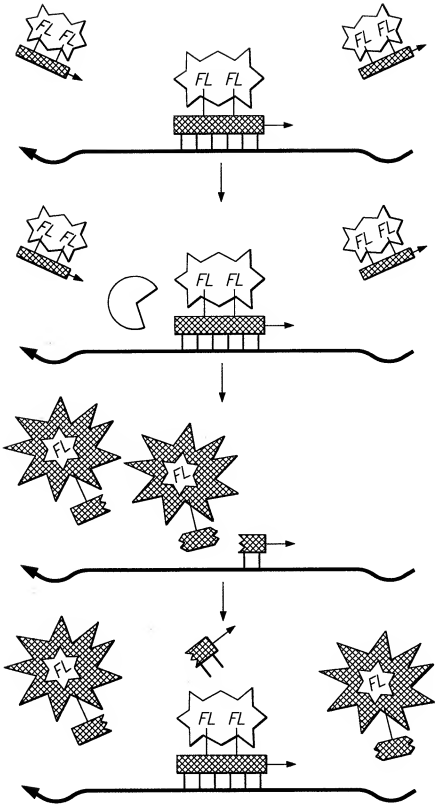


FIG. 27

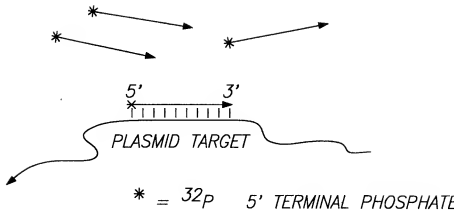


FIG. 28A

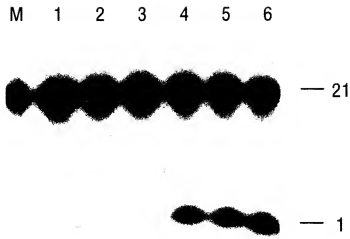


FIG. 28B

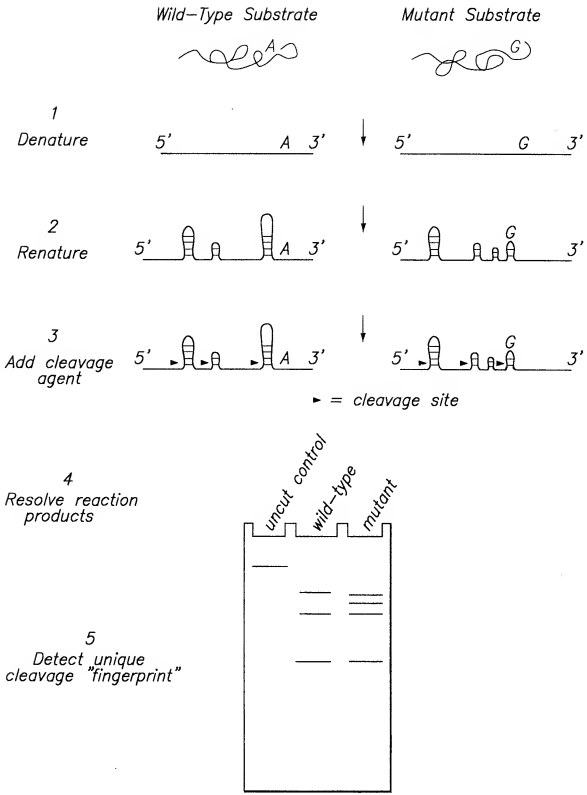

Wild–Type Substrate
Mutant Substrate
A
G
1
Denature
5' A 3'
5' G 3'
2
Renature
5' A 3'
5' G 3'
3
Add cleavage agent
5' A 3'
5' G 3'
► = cleavage site
4
Resolve reaction products
uncut control
wild–type
mutant
5
Detect unique cleavage "fingerprint"

FIG. 29

FIG. 30

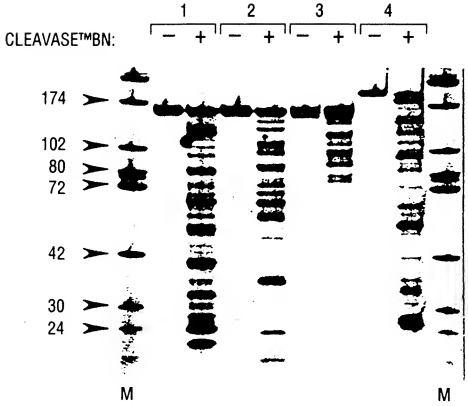


FIG. 31

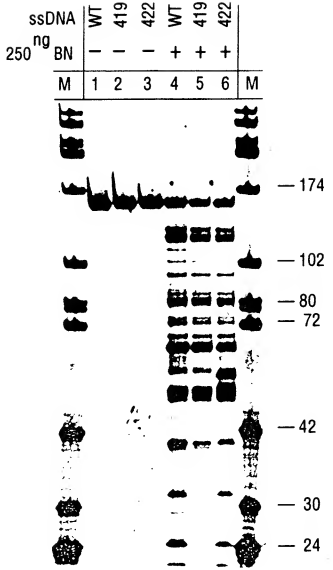

ssDNA
250 ng BN
WT
419
422
WT
419
422
M
1
2
3
4
5
6
M
174
102
80
72
42
30
24

FIG. 32

FIG. 33

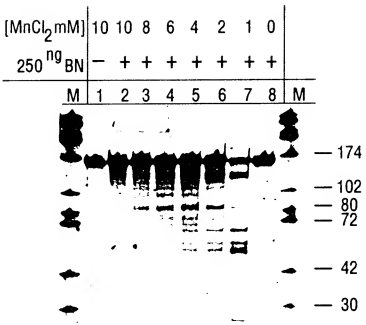


FIG. 34

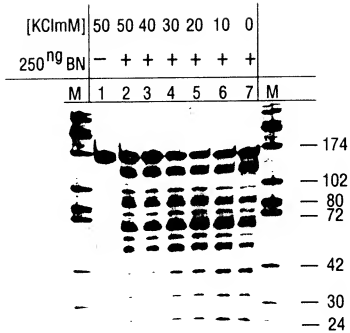

[KClmM] 50 50 40 30 20 10 0
250 ng BN − + + + + + +
M 1 2 3 4 5 6 7 M
174
102
80
72
42
30
24

FIG. 35

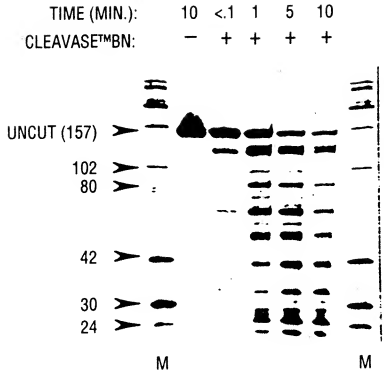


FIG. 36

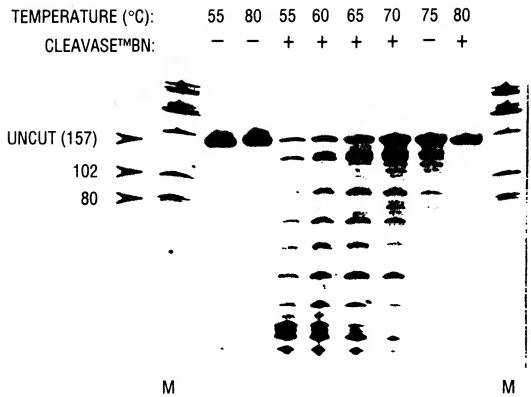

TEMPERATURE (°C): 55 80 55 60 65 70 75 80
CLEAVASE™BN: − − + + + + − +
UNCUT (157)
102
80
M
M

FIG. 37

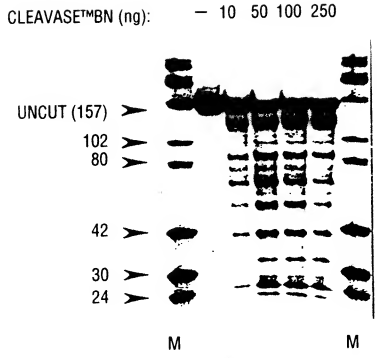


FIG. 38

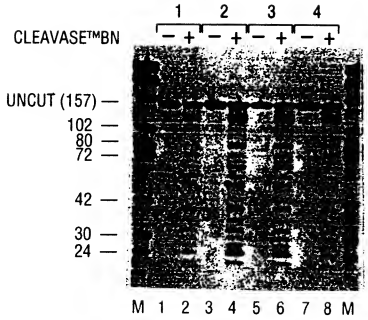


FIG. 39

STRAND	5' - BIOTIN SENSE STRAND						5' - FLUORESCEIN ANTI-SENSE STRAND					
ssDNA	WT	419	422	WT	419	422	WT	419	422	WT	419	422
250 ng BN	−	−	−	+	+	+	+	+	+	−	−	−
M	1	2	3	4	5	6	7	8	9	10	11	12

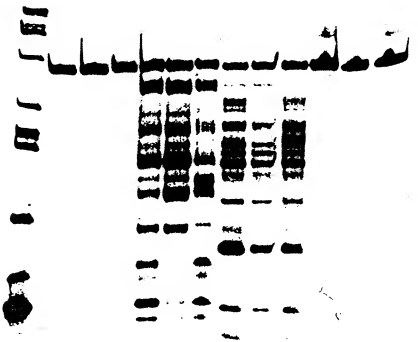


FIG. 40

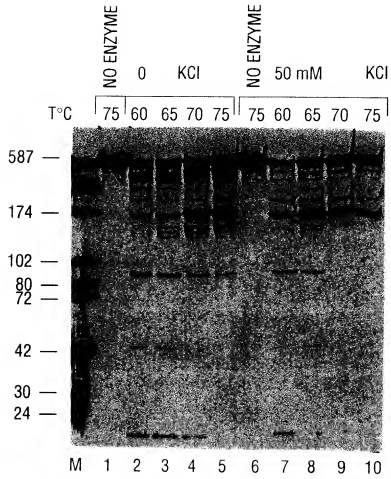

NO ENZYME
0 KCl
NO ENZYME
50 mM KCl
T°C
75 60 65 70 75 75 60 65 70 75
587
174
102
80
72
42
30
24
M 1 2 3 4 5 6 7 8 9 10

FIG. 41

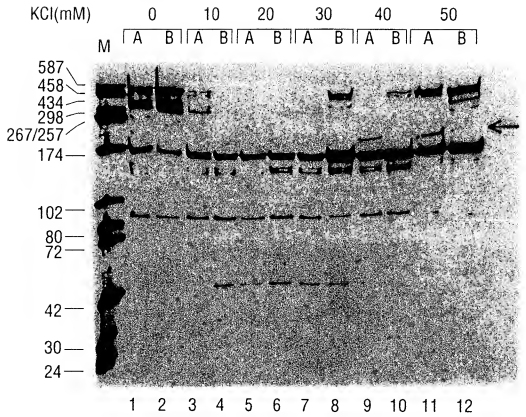

KCl(mM)
0
10
20
30
40
50
A B A B A B A B A B A B
M
587
458
434
298
267/257
174
102
80
72
42
30
24
1 2 3 4 5 6 7 8 9 10 11 12

FIG. 42

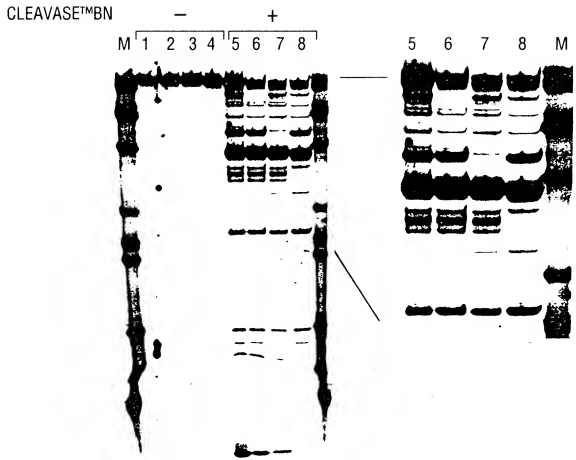


FIG. 43

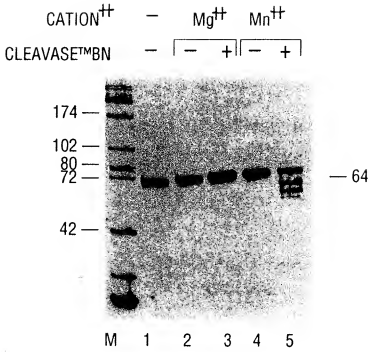


FIG. 44

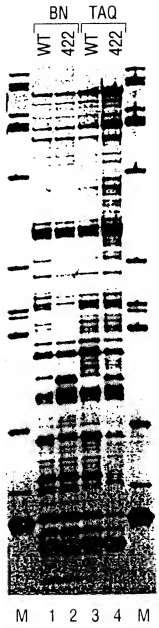


FIG. 45

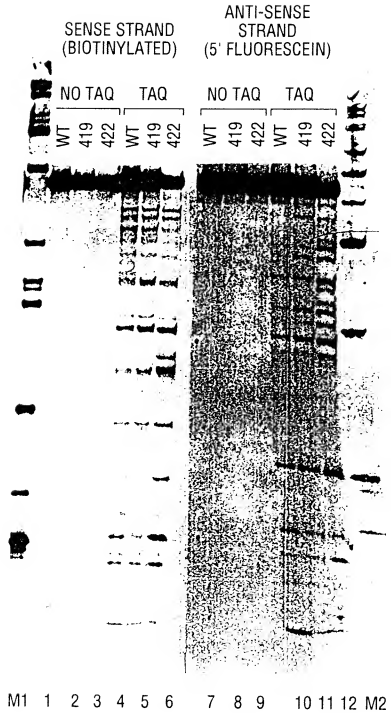

SENSE STRAND
(BIOTINYLATED)
ANTI-SENSE
STRAND
(5' FLUORESCEIN)
NO TAQ
TAQ
NO TAQ
TAQ
WT
419
422
WT
419
422
WT
419
422
WT
419
422
M1 1 2 3 4 5 6 7 8 9 10 11 12 M2

FIG. 46

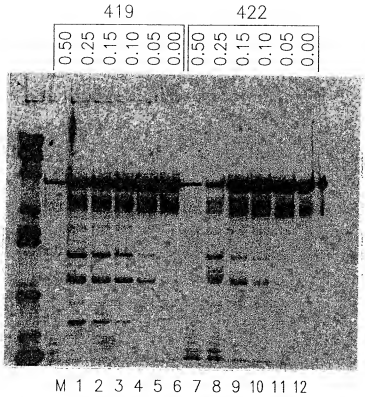

419
422
0.50
0.25
0.15
0.10
0.05
0.00
0.50
0.25
0.15
0.10
0.05
0.00
M 1 2 3 4 5 6 7 8 9 10 11 12

FIG. 47

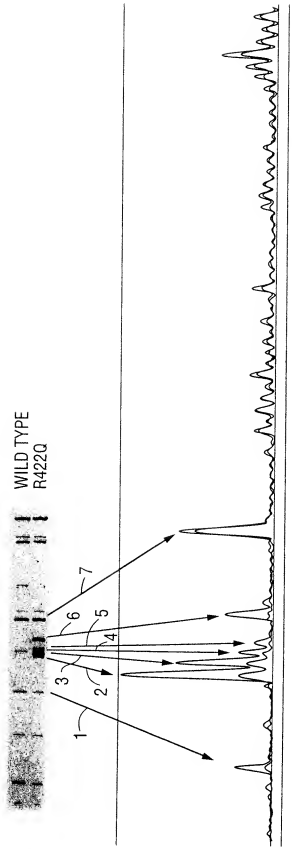


FIG. 48

L.100.8-1	5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGACTTTCCACAAGGGG
(SEQ ID NO: 76)	3'CCGACTGTTCTTCCTTTGAGCGACTCTGTCGTCCCTGAAAGGTGTTCCCC
L.46.16-10	5'GGCTGACAAGAAGGAAACTCGCTGAGATAGCAGGGACTTTCCACAAGGGG
(SEQ ID NO: 77)	3'CCGACTGTTCTTCCTTTGAGCGACTCTATCGTCCCTGAAAGGTGTTCCCC
L.46.16-12	5'GGCTGACAAGAAGGAAACTCGCTGAGATAGCAGGGACTTTCCACAAGGGG
(SEQ ID NO: 78)	3'CCGACTGTTCTTCCTTTGAGCGACTCTATCGTCCCTGAAAGGTGTTCCCC
L19.16-3	5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGACTTTCCACAAGGGG
(SEQ ID NO: 79)	3'CCGACTGTTCTTCCTTTGAGCGACTCTGTCGTCCCTGAAAGGTGTTCCCC
L.CEM/251	5'GGCTGACAAGAAGGAAACTCGCTGAAACAGCAGGGACTTTCCACAAGGGG
(SEQ ID NO: 80)	3'CCGACTGTTCTTCCTTTGAGCGACTTTGTCGTCCCTGAAAGGTGTTCCCC
L.36.8-3	5'GGCTGACAAGAAGGAAACTCGCTGAGACAGCAGGGACTTTCCACAAGGGG
(SEQ ID NO: 81)	3'CCGACTGTTCTTCCTTTGAGCGACTCTGTCGTCCCTGAAAGGTGTTCCCC

FIG. 49A

```
                                                                                                    100
L.100.8-1           ATGTTACGGGGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCACTCTCT
(SEQ ID NO: 76)     TACAATGCCCCTCCATGACCCCTCCTCGGCCAGCCCTTGCGGGTGAGAGA

L.46.16-10          ATGTTATGGGGAGG-----------AGCCGGTCGGGAACACCCACTTTCT
(SEQ ID NO: 77)     TACAATACCCCTCC-----------TCGGCCAGCCCTTGTGGGTGAAAGA

L.46.16-12          ATGTTATGGGGAGG-----------AGCCGGTCGGGAACACCCACTTTCT
(SEQ ID NO: 78)     TACAATACCCCTCC-----------TCGGCCAGCCCTTGTGGGTGAAAGA

L19.16-3            ATGTTACGGGGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCCCTCTCT
(SEQ ID NO: 19)     TACAATGCCCCTCCATGACCCCTCCTCGGCCAGCCCTTGCGGGGGAGAGA

L.CEM/251           ATGTTACGGGGAGGTACTGGGAAGGAGCCGGTCGGGAACGCCCACTTTCT
(SEQ ID NO: 80)     TACAATGCCCCTCCATGACCCTTCCTCGGCCAGCCCTTGCGGGTGAAAGA

L.36.8-3            ATGTTACGGAGAGGTACTGGGGAGGAGCCGGTCGGGAACGCCCACTCTCT
(SEQ ID NO: 81)     TACAATGCCTCTCCATGACCCCTCCTCGGCCAGCCCTTGCGGGTGAGAGA
```

FIG. 49B

150

L.100.8-1

5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA
3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT

L.46.16-10

5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA
3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT

L.46.16-12

5'TGGTGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA
3'ACCACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT

L.19.16-3

5'TGATGTATAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA
3'ACTACATATTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT

L.CEM/251

5'TGATGTATAAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA
3'ACTACATATTTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT

L.36.8-3

5'TGATGTATAAAATATCACTGCATTTCGCTCTGTATTCAGTCGCTCTGCGGA
3'ACTACATATTTTATAGTGACGTAAAGCGAGACATAAGTCAGCGAGACGCCT

FIG. 49C

```
                                                                    200
L.100.8-1       GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
                CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.46.16-10      GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGCTAG
                CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.46.16-12      GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
                CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.19.16-3       GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
                CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.CEM/251       GAGGCTGGCAGATTGAGCCCTGGGAGGTTCTCTCCAGCACTAGCAGGTAG
                CTCCGACCGTCTAACTCGGGACCCTCCAAGAGAGGTCGTGATCGTCCATC

L.36.8-3        GAGGCTGGCAGATTGAGCCCTAGGAGGTTCTCTCCAGCACTAGCAGGTAG
                CTCCGACCGTCTAACTCGGGATCCTCCAAGAGAGGTCGTGATCGTCCATC
```

FIG. 49D

```
                                                            250
L. 100. 8 -1        5'AGCCTGGGTGTTCCCTGCTAGACTCTCACCAGCACTTGGCCGGTGCTGGG
(SEQ ID NO: 76)     3'TCGGACCCACAAGGGACCATCTGAGAGTGGTCGTGAACCGGCCACGACCC

L. 46.16-10         5'AGCCTGGGTGTTCCCTGCTAGACTCTCACCAGCACTTAGCCAGTGCTGGG
(SEQ ID NO: 77)     3'TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAATCGGTCACGACCC

L. 46.16-12         5'AGCCTGGGTGTTCCCTGCTAGACTCTCACCAGCACTTGGCCAGTGCTGGG
(SEQ ID NO: 78)     3'TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAACCGGTCACGACCC

L. 19.16-3          5'AGCCTGGGTGTTCCCTGCTAGACTCTCACCAGCACTTGGCCGGTGCTGGG
(SEQ ID NO: 79)     3'TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAACCGGCCACGACCC

L. CEM/251          5'AGCCTGGGTGTTCCCTGCTAGACTCTCACCAGCACTTGGCCGGTGCTGGG
(SEQ ID NO: 80)     3'TCGGACCCACAAGGGACGATCTGAGAGTGGTCGTGAACCGGCCACGACCC

L. 36.8-3           5'AGCCTGAGTGTTCCCTGCTAAACTCTCACCAGCACTTGGCCGGTGCTGGG
(SEQ ID NO: 81)     3'TCGGACTCACAAGGGACGATTTGAGAGTGGTCGTGAACCGGCCACGACCC

                                                     HAIRPIN
```

FIG. 49E

300
L. 100. 8 -1
(SEQ ID NO: 76)
CAGAGTGGCTCCACGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
GTCTCACCGAGGTGCGAACGAATTTCTGGAGAAGTTATTTCGACGC
L. 46.16-10
(SEQ ID NO: 77)
CAGAGTGGCTCCACGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
GTCTCACCGAGGTGCGAACGAATTTCTGGAGAAGTTATTTCGACGG
L. 46.16-12
(SEQ ID NO: 78)
CAGAGTGGCTCCACGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
GTCTCACCGAGGTGCGAACGAATTTCTGGAGAAGTTATTTCGACGG
L. 19.16-3
(SEQ ID NO: 79)
CAGAGTGGCTCCACGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
GTCTCACCGAGGTGCGAACGAATTTCTGGAGAAGTTATTTCGACGG
L. CEM/251
(SEQ ID NO: 80)
CAGAGTGACTCCACGCTTGCTTAAAGCCCTCTTCAATAAAGCTGCC
GTCTCACTGAGGTGCGAACGAATTTCGGGAGAAGTTATTTCGACGG
L. 36.8-3
(SEQ ID NO: 81)
CAGAGCGGCTCCACGCTTGCTTAAAGACCTCTTCAATAAAGCTGCC
GTCTCGCCGAGGTGCGAACGAATTTCTGGAGAAGTTATTTCGACGG
HAIRPIN

FIG. 49F


350
L.100.8-1
5'ATTTTAGAAGTAGGCCAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATCCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'
L.46,16-10
5'ATTTTAGAAGTAAGCCAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATTCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'
L.46.16-12
5'ATTTTAGAAGTAAGCCAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATTCGGTCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'
L.19.16-3
5'ATTTTAGAAGTAGGCTAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATCCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'
L.CEM/251
5'ATTTTAGAAGTAAGCTAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATTCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'
L.36.8-3
5'ATTTTAGAAGTAGGCTAGTGTGTGTTCCCATCTCTCCTAGCCGCCGCCTG G 3'
3'TAAAATCTTCATCCGATCACACACAAGGGTAGAGAGGATCGGCGGCGGAC C 5'

FIG. 49G

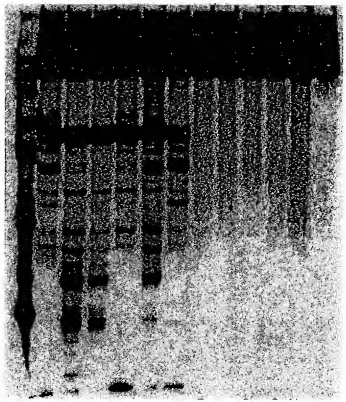


FIG. 50

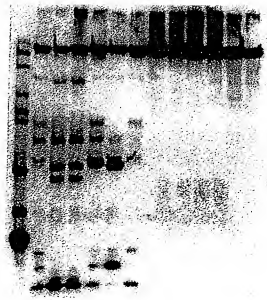


FIG. 51

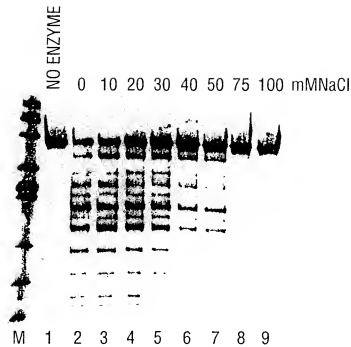


FIG. 52

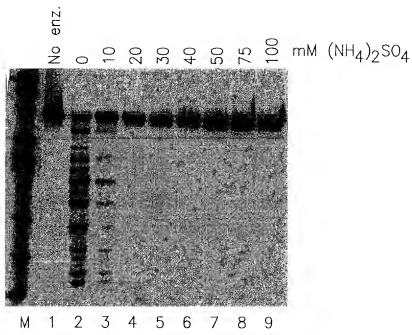

No enz.
0
10
20
30
40
50
75
100
mM $(NH_4)_2SO_4$
M 1 2 3 4 5 6 7 8 9

FIG. 53

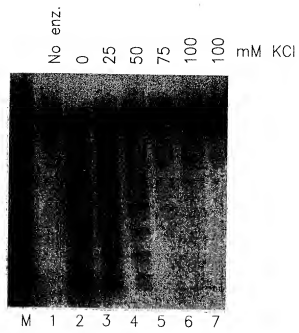

No enz.
0
25
50
75
100
100
mM KCl
M 1 2 3 4 5 6 7

FIG. 54

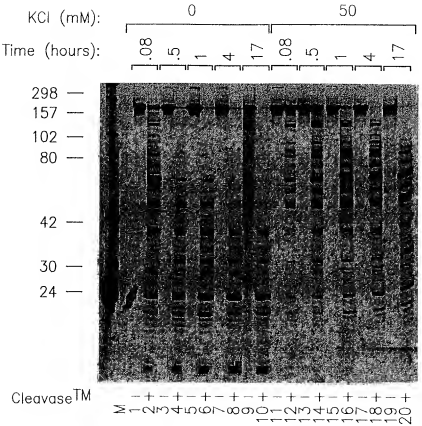

KCl (mM):
0
50
Time (hours):
.08
.5
1
4
17
.08
.5
1
4
17
298
157
102
80
42
30
24
Cleavase™
M
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20

FIG. 55

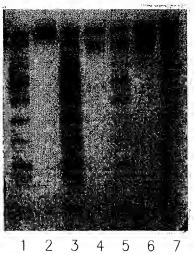

1 2 3 4 5 6 7

FIG. 56

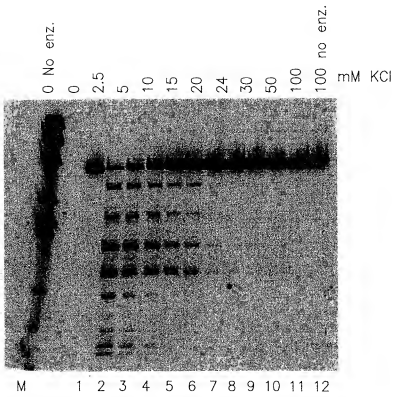

0 No enz.
0
2.5
5
10
15
20
24
30
50
100
100 no enz.
mM KCl
M
1 2 3 4 5 6 7 8 9 10 11 12

FIG. 57

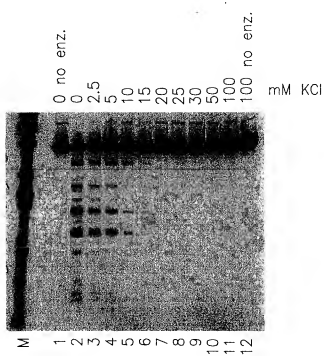

0 no enz.
0
2.5
5
10
15
20
25
30
50
100
100 no enz.
mM KCl
M
1 2 3 4 5 6 7 8 9 10 11 12

FIG. 58

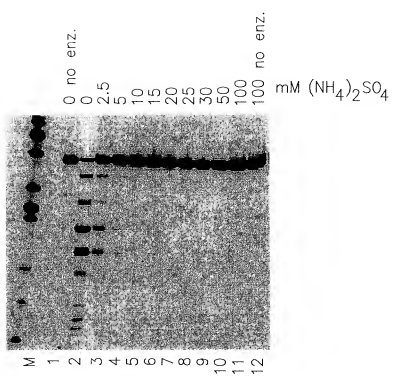

0 no enz.
0
2.5
5
10
15
20
25
30
50
100
100 no enz.
mM $(NH_4)_2SO_4$
M
1
2
3
4
5
6
7
8
9
10
11
12

FIG. 59

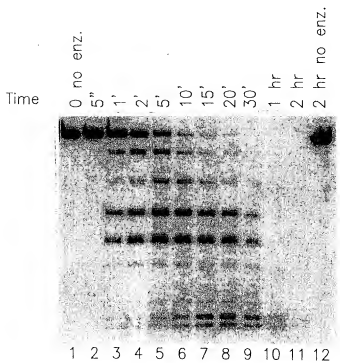

Time
0 no enz.
5"
1'
2'
5'
10'
15'
20'
30'
1 hr
2 hr
2 hr no enz.
1 2 3 4 5 6 7 8 9 10 11 12

FIG. 60

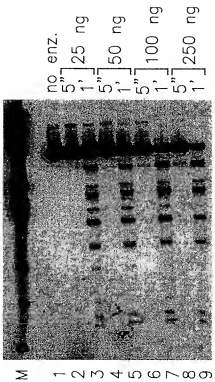

no enz.
5"
1'
25 ng
5"
1'
50 ng
5"
1'
100 ng
5"
1'
250 ng
Cleavase™ BN
Time
M
1
2
3
4
5
6
7
8
9

FIG. 61

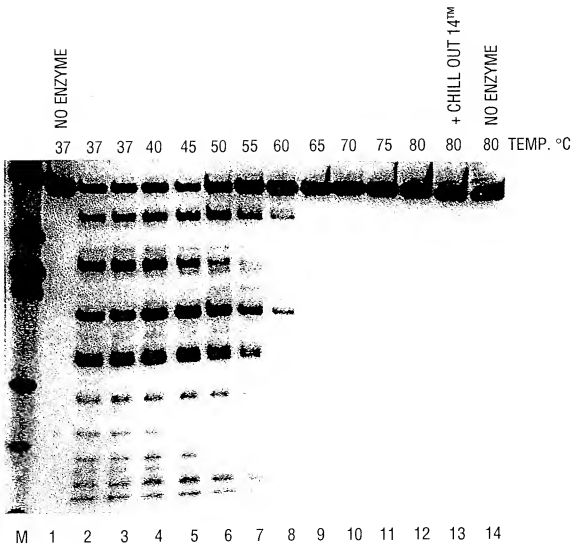

NO ENZYME
+ CHILL OUT 14™
NO ENZYME
37 37 37 40 45 50 55 60 65 70 75 80 80 80 TEMP. °C
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIG. 62

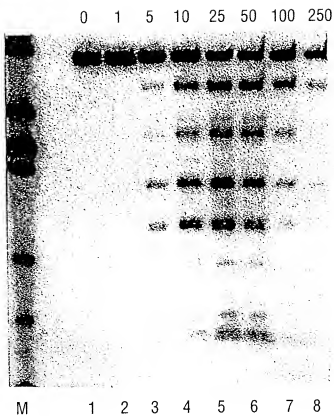


FIG. 63

FIG. 64A

FIG. 64B

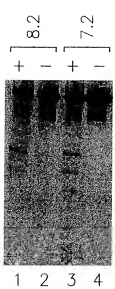


FIG. 65A

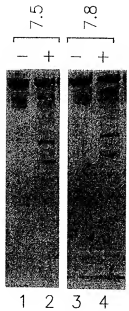


FIG. 65B

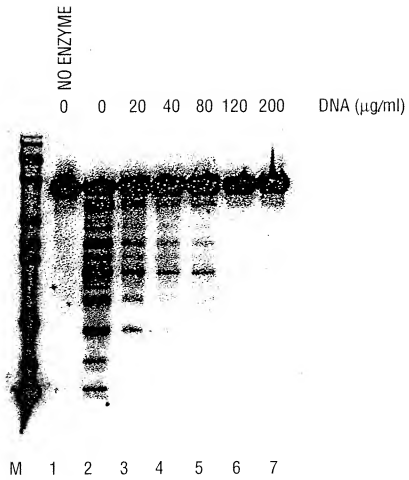


FIG. 66

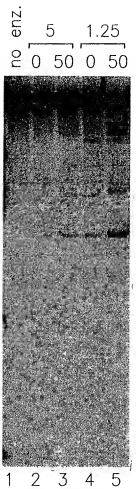

no enz.
5
1.25
units Tfl DNAP
0 50 0 50
mM KCl
1 2 3 4 5

FIG. 67

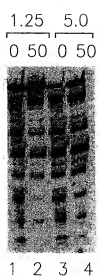

1.25
5.0
units Tth DNAP
0 50 0 50
mM KCl
1 2 3 4

FIG. 68

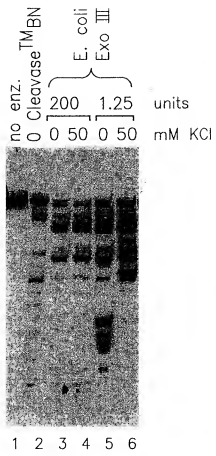

no enz.
0 Cleavase™BN
E. coli Exo III
200 1.25 units
0 50 0 50 mM KCl
1 2 3 4 5 6

FIG. 69

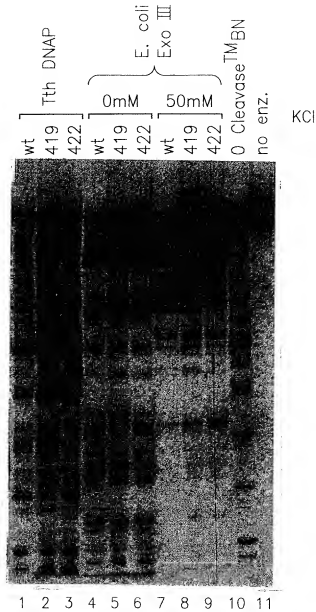
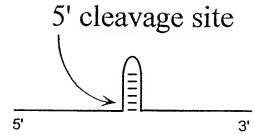
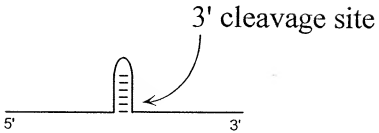

Tth DNAP
E. coli Exo III
0mM 50mM
KCl
wt 419 422 wt 419 422 wt 419 422 0 Cleavase™BN no enz.
1 2 3 4 5 6 7 8 9 10 11

FIG. 70

FIG. 71





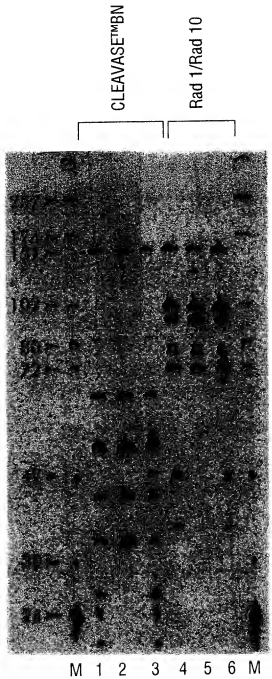


FIG. 72

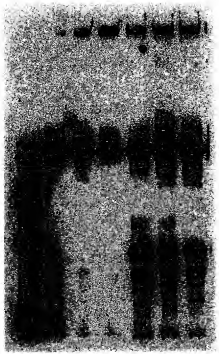


FIG. 73

FIG. 74A

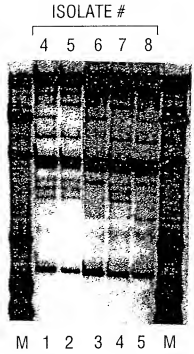


FIG. 74B

FIG. 75

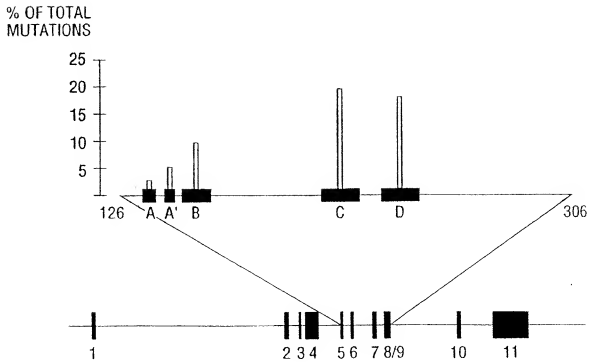


FIG. 76

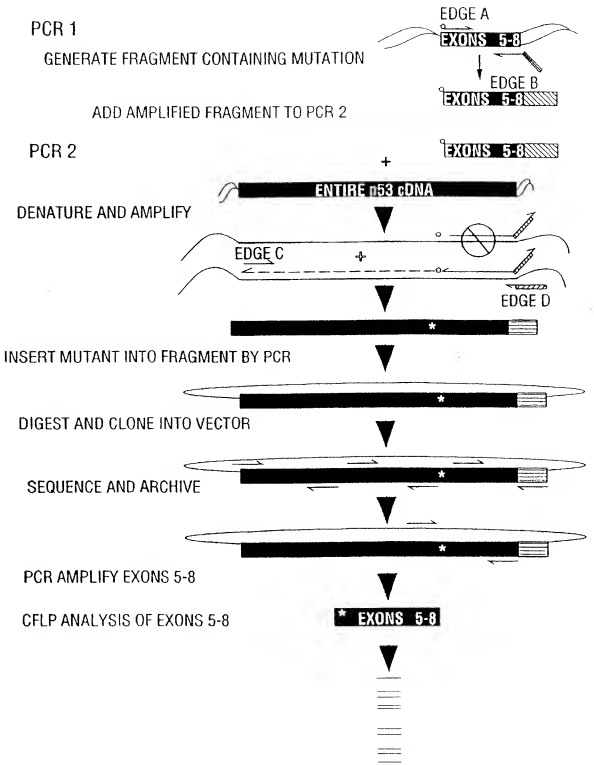

PCR 1
GENERATE FRAGMENT CONTAINING MUTATION
EDGE A
EXONS 5-8
EDGE B
EXONS 5-8
ADD AMPLIFIED FRAGMENT TO PCR 2
PCR 2
EXONS 5-8
+
ENTIRE p53 cDNA
DENATURE AND AMPLIFY
EDGE C
EDGE D
INSERT MUTANT INTO FRAGMENT BY PCR
DIGEST AND CLONE INTO VECTOR
SEQUENCE AND ARCHIVE
PCR AMPLIFY EXONS 5-8
CFLP ANALYSIS OF EXONS 5-8
* EXONS 5-8

FIG. 77

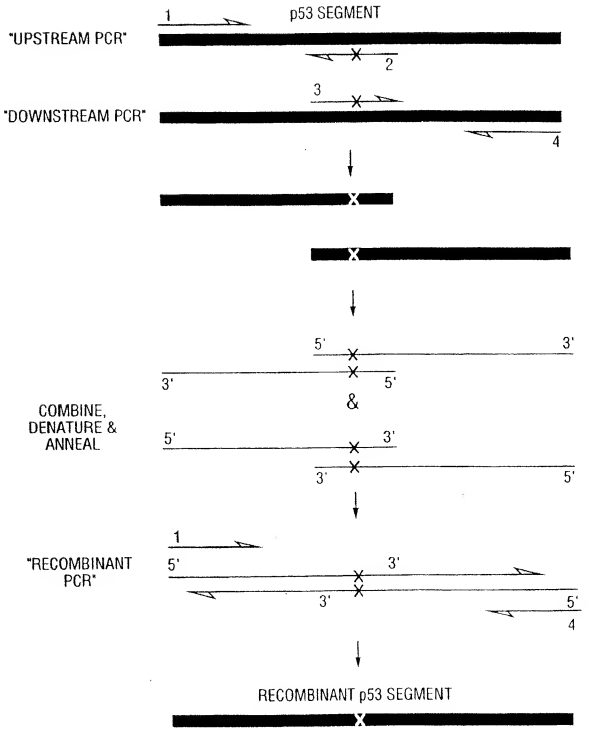

1
p53 SEGMENT
"UPSTREAM PCR"
2
3
"DOWNSTREAM PCR"
4
5'
3'
3'
5'
&
COMBINE, DENATURE & ANNEAL
5'
3'
3'
5'
1
"RECOMBINANT PCR"
5'
3'
3'
5'
4
RECOMBINANT p53 SEGMENT

FIG. 78

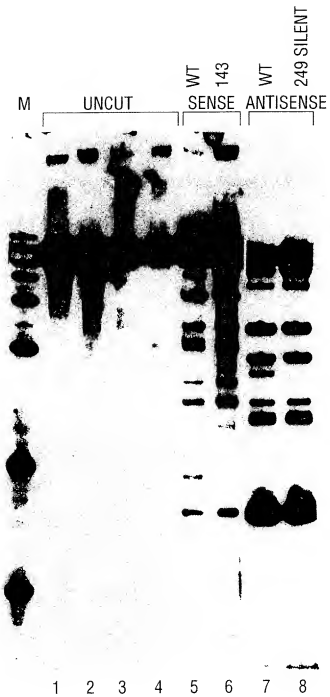


FIG. 79

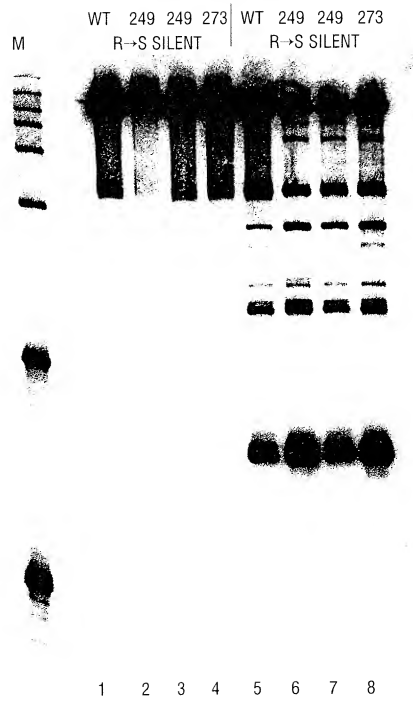


FIG. 80

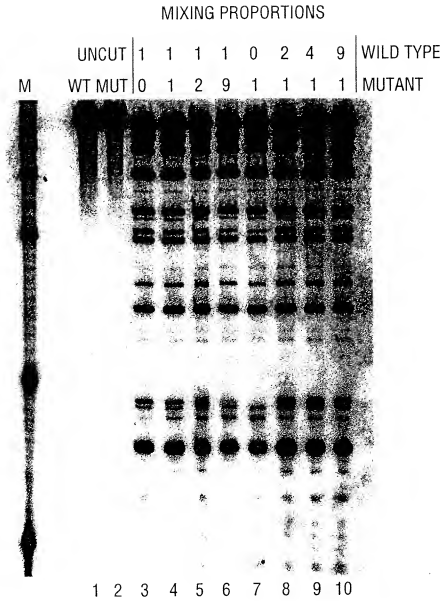

MIXING PROPORTIONS
UNCUT 1 1 1 1 0 2 4 9 WILD TYPE
M WT MUT 0 1 2 9 1 1 1 1 MUTANT
1 2 3 4 5 6 7 8 9 10

FIG. 81

FIG. 82

```
HCV1.1 (SEQ ID NO:121)      1 CTGTCTTCAC GCAGAAAGCG TCTGGCCATG GCGTTAGTAT GAGTGTCGTG 50
HCV2.1 (SEQ ID NO:122)        CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV3.1 (SEQ ID NO:123)        CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV4.2 (SEQ ID NO:124)        CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTG
HCV6.1 (SEQ ID NO:125)        CTGTCTTCAC GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTCGTA
HCV7.1 (SEQ ID NO:126)        CTGTCTTCAC GCAGAAAGCG CCTAGCCATG GCGTTAGTAC GAGTGTCGTG

HCV1.1                       51 CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC 100
HCV2.1                          CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV3.1                          CAGCCTCCAG GTCCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV4.2                          CAGCCTCCAG GACCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV6.1                          CAGCCTCCAG GCCCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC
HCV7.1                          CAGCCTCCAG GACCCCCCT CCCGGGAGAG CCATAGTGGT CTGCGGAACC

HCV1.1                      101 GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-AAA 150
HCV2.1                          GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-CAA
HCV3.1                          GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC TTGGAT-CAA
HCV4.2                          GGTGAGTACA CCGGAATTGC CAGGACGACC GGGTCCTTTC GTGGATGTAA
HCV6.1                          GGTGAGTACA CCGGAATTGC CGGGAAGACT GGGTCCTTTC TTGGAT-AAA
HCV7.1                          GGTGAGTACA CCGGAATCGC TGGGGTGACC GGGTCCTTTC TTGGAG-CAA

HCV1.1                      151 CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG 200
HCV2.1                          CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV3.1                          CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCGAGA CTGCTAGCCG
HCV4.2                          CCCGCTCAAT GCCTGGAGAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV6.1                          CCCACTCTAT GCCCGGCCAT TTGGGCGTGC CCCCGCAAGA CTGCTAGCCG
HCV7.1                          CCCGCTCAAT ACCCAGAAAT TTGGGCGTGC CCCCGCGAGA TCACTAGCCG

HCV1.1                      201 AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCCT 250
HCV2.1                          AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV3.1                          AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV4.2                          AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV6.1                          AGTAGCGTTG GGTTGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT
HCV7.1                          AGTAGTGTTG GGTCGCGAAA GGCCTTGTGG TACTGCCTGA TAGGGTGCTT

HCV1.1                      251 GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC 282
HCV2.1                          GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV3.1                          GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV4.2                          GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
HCV6.1                          GCGAGTACCC CGGGAGGTCT CGTAGACCGT GC
HCV7.1                          GCGAGTGCCC CGGGAGGTCT CGTAGACCGT GC
```

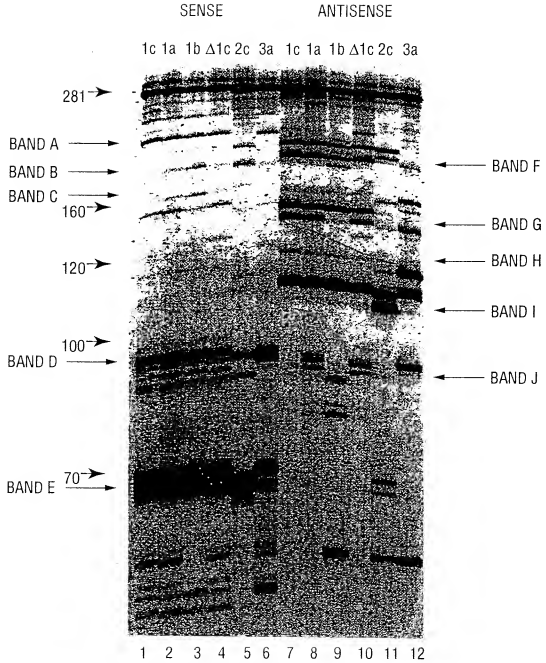

SENSE
ANTISENSE
1c 1a 1b Δ1c 2c 3a 1c 1a 1b Δ1c 2c 3a
281
BAND A
BAND B
BAND C
160
120
100
BAND D
70
BAND E
BAND F
BAND G
BAND H
BAND I
BAND J
1 2 3 4 5 6 7 8 9 10 11 12

FIG. 83

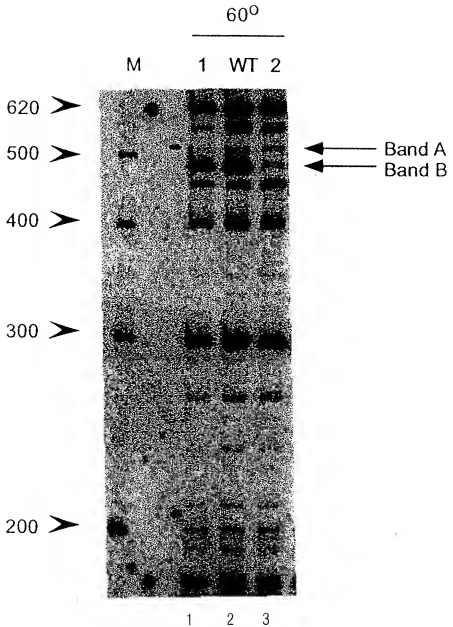


FIG. 84

FIG. 85A

FIG. 85B

SENSE STRAND

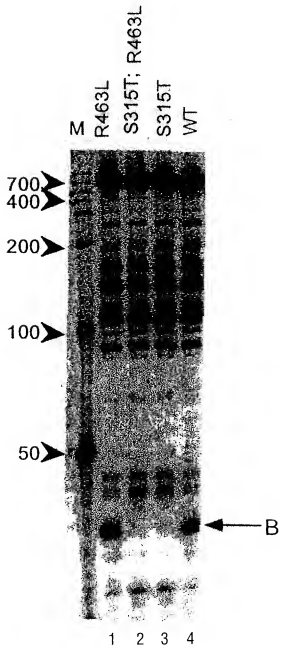


FIG. 86

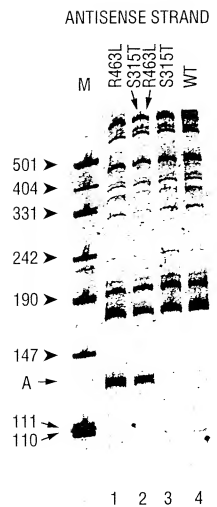

ANTISENSE STRAND
M
R463L
S315T
R463L
S315T
WT
501
404
331
242
190
147
A
111
110
1
2
3
4

FIG. 87

```
        10         20         30         40         50         60
         AGA GTTTGATCCT GGCTCAG                                        1638
AAATTGAAGA GTTTGATCAT GGCTCAGATT GAACGCTGGC GGCAGGCCTA ACACATGCAA
TTTAACTTCT CAAACTAGTA CCGAGTCTAA CTTGCGACCG CCGTCCGGAT TGTGTACGTT

        70         80         90        100        110        120
                                                GGCGGAC GGGTGAGTAA      ER10
GTCGAACGGT AACAGGAAGA AGCTTGCTTC TTTGCTGACG AGTGGCGGAC GGGTGAGTAA
CAGCTTGCCA TTGTCCTTCT TCGAACGAAG AAACGACTGC TCACCGCCTG CCCACTCATT

       130        140        150        160        170        180
TGTCTGGGAA ACTGCCTGAT GGAGGGGGAT AACTACTGGA AACGGTAGCT AATACCGCAT
ACAGACCCTT TGACGGACTA CCTCCCCCTA TTGATGACCT TTGCCATCGA TTATGGCGTA

       190        200        210        220        230        240
AACGTCGCAA GACCAAAGAG GGGGACCTTC GGGCCTCTTG CCATCGGATG TGCCCAGATG
TTGCAGCGTT CTGGTTTCTC CCCCTGGAAG CCCGGAGAAC GGTAGCCTAC ACGGGTCTAC

       250        260        270        280        290        300
GGATTAGCTA GTAGGTGGGG TAACGGCTCA CCTAGGCGAC GATCCCTAGC TGGTCTGAGA
CCTAATCGAT CATCCACCCC ATTGCCGAGT GGATCCGCTG CTAGGGATCG ACCAGACTCT

       310        320        330        340        350        360
GGATGACCAG CCACACTGGA ACTGAGACAC GGTCCAGACT CCTACGGGAG GCAGCAGTGG
CCTACTGGTC GGTGTGACCT TGACTCTGTG CCAGGTCTGA GGATGCCCTC CGTCGTCACC
                                    TGA GGATGCCCTC CGTCGTC         1659
```

FIG. 88A

```
          370       380       390       400       410       420
GGAATATTGC ACAATGGGCG CAAGCCTGAT GCAGCCATGC CGCGTGTATG AAGAAGGCCT
CCTTATAACG TGTTACCCGC GTTCGGACTA CGTCGGTACG GCGCACATAC TTCTTCCGGA

          430       440       450       460       470       480
TCGGGTTGTA AAGTACTTTC AGCGGGGAGG AAGGGAGTAA AGTTAATACC TTTGCTCATT
AGCCCAACAT TTCATGAAAG TCGCCCCTCC TTCCCTCATT TCAATTATGG AAACGAGTAA

          490       500       510       520       530       540
GACGTTACCC GCAGAAGAAG CACCGGCTAA CTCCGTGCCA GCAGCCGCGG TAATACGGAG
CTGCAATGGG CGTCTTCTTC GTGGCCGATT GAGGCACGGT CGTCGGCGCC ATTATGCCTC

          550       560       570       580       590       600
GGTGCAAGCG TTAATCGGAA TTACTGGGCG TAAAGCGCAC GCAGGCGGTT TGTTAAGTCA
CCACGTTCGC AATTAGCCTT AATGACCCGC ATTTCGCGTG CGTCCGCCAA ACAATTCAGT

          610       620       630       640       650       660
GATGTGAAAT CCCCGGGCTC AACCTGGGAA CTGCATCTGA TACTGGCAAG CTTGAGTCTC
CTACACTTTA GGGGCCCGAG TTGGACCCTT GACGTAGACT ATGACCGTTC GAACTCAGAG

          670       680       690       700       710       720
GTAGAGGGGG GTAGAATTCC AGGTGTAGCG GTGAAATGCG TAGAGATCTC GAGGAATACC
CATCTCCCCC CATCTTAAGG TCCACATCGC CACTTTACGC ATCTCTAGAC CTCCTTATGG

          730       740       750       760       770       780
GGTGGCGAAG GCGGCCCCCT GGACGAAGAC TGACGCTCAG GTGCGAAAGC GTGGGGAGCA
CCACCGCTTC CGCCGGGGGA CCTGCTTCTG ACTGCGAGTC CACGCTTTCG CACCCTCGT
```

FIG. 88B

```
          790                 800                 810                 820                 830                 840
AACAGGATTA GATACCCTGG TAGTCCACGC CGTAAACGAT GTCGACTTGG AGGTTGTGCC
TTGTCCTAAT CTATGGGACC ATCAGGTGCG GCATTTGCTA CAGCTGAACC TCCAACACGG

          850                 860                 870                 880                 890                 900
CTTGAGGCGT GGCTTCCGGA GCTAACGCGT TAAGTCGACC GCCTGGGGAG TACGGCCGCA
GAACTCCGCA CCGAAGGCCT CGATTGCGCA ATTCAGCTGG CGGACCCCTC ATGCCGGCGT

          910                 920                 930                 940                 950                 960
AGGTTAAAAC TCAAATGAAT TGACGGGGGC CCGCACAAGC GGTGGAGCAT GTGGTTTAAT
TCCAATTTTG AGTTTACTTA ACTGCCCCCG GGCGTGTTCG CCACCTCGTA CACCAAATTA

          970                 980                 990                1000                1010                1020
TCGATGCAAC GCGAAGAACC TTACCTGGTC TTGACATCCA CGGAAGTTTT CAGAGATGAG
AGCTACGTTG CGCTTCTTGG AATGGACCAG AACTGTAGGT GCCTTCAAAA GTCTCTACTC

         1030                1040                1050                1060                1070                1080
AATGTGCCTT CGGGAACCGT GAGACAGGTG CTGCATGGCT GTCGTCAGCT CGTGTTGTGA
TTACACGGAA GCCCTTGGCA CTCTGTCCAC GACGTACCGA CAGCAGTCGA GCACAACACT

         1090                1100                1110                1120                1130                1140
                              GC AACGAGCGCA ACCC                                                              SB-1

AATGTTGGGT TAAGTCCCGC AACGAGCGCA ACCCTTATCC TTTGTTGCCA GCGGTCCGGC
TTACAACCCA ATTCAGGGCG TTGCTCGCGT TGGGAATAGG AAACAACGGT CGCCAGGCCG

         1150                1160                1170                1180                1190                1200
                                                                                          ATG ACGTCAAGTC                SB-3
                                                                                          ATG ACGTCAAGTC                SB-4

CGGGAACTCA AAGGAGACTG CCAGTGATAA ACTGGAGGAA GGTGGGGATG ACGTCAAGTC
GCCCTTGAGT TTCCTCTGAC GGTCACTATT TGACCTCCTT CCACCCCTAC TGCAGTTCAG
```

FIG. 88C

```
      1210        1220        1230        1240        1250        1260
ATCATGGCCC TTA                                                          SB-3
ATCATGGCCC TTACGA                                                       SB-4
ATCATGGCCC TTACGACCAG GGCTACACAC GTGCTACAAT GGCGCATACA AAGAGAAGCG
TAGTACCGGG AATGCTGGTC CCGATGTGTG CACGATGTTA CCGCGTATGT TTCTCTTCGC

      1270        1280        1290        1300        1310        1320
ACCTCGCGAG AGCAAGCGGA CCTCATAAAG TGCGTCGTAG TCCGGATTGG AGTCTGCAAC
TGGAGCGCTC TCGTTCGCCT GGAGTATTTC ACGCAGCATC AGGCCTAACC TCAGACGTTG

      1330        1340        1350        1360        1370        1380
TCGACTCCAT GAAGTCGGAA TCGCTAGTAA TCGTGGATCA GAATGCCACG GTGAATACGT
AGCTGAGGTA CTTCAGCCTT AGCGATCATT AGCACCTAGT CTTACGGTGC CACTTATGCA
                                                    GC CACTTATGCA       1743

      1390        1400        1410        1420        1430        1440
TCCCGGGCCT TGTACACACC GCCCGTCACA CCATGGGAGT GGGTTGCAAA AGAAGTAGGT
AGGGCCCGGA ACATGTGTGG CGGGCAGTGT GGTACCCTCA CCCAACGTTT TCTTCATCCA
AGGGCCCGGA ACATG                                                        1743

      1450        1460        1470        1480        1490        1500
AGCTTAACCT TCGGGAGGGC GCTTACCACT TTGTGATTCA TGACTGGGGT GAAGTCGTAA
TCGAATTGGA AGCCCTCCCG CGAATGGTGA AACACTAAGT ACTGACCCCA CTTCAGCATT

      1510        1520        1530        1540        1550
CAAGGTAACC GTAGGGGAAC CTGCGGTTGG ATCACCTCCT TA.......
GTTCCATTGG CATCCCCTTG GACGCCAACC TAGTGGAGGA AT.......
```

FIG. 88D

```
1638 (SEQ ID NO:151)                    AGAGTTTGATCCTGGCTCAG
E.colirrsE(SEQ ID NO:158)0 ...AAATTGAAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCA
Cam.jejun5(SEQ ID NO:159)0 ~TTTTTATGGAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCGTGCCTAATACATGCA
Stp.aureus(SEQ ID NO:160)0 ..TTTTATGGAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCA

ER10 (SEQ ID NO:152)                                                    GGCGGACGGG
E.colirrsE                60 AGTCGAACGGTAACAG----GAAGAAGCTTGCTTCTTT----GCTGACGAGTGGCGGACGGG
Cam.jejun5                62 AGTCGAACGAT-----GAAGCTTCTAGCTTGCTAGAAGTGGA-----TTAGTGGCGCACGGG
Stp.aureus                61 AGTCGAGCGAA-----CGGACGAGAAGCTTGCTTCTCTGATG----TT-AGCGGCGGACGGG

ER10                         TGAGTAA
E.colirrsE               114 TGAGTAATGTCTGGGA-AACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATA
Cam.jejun5               114 TGAGTAAGGTATAGTTAATCTGCCCTACACAAGAGGACAACAGTTGGAAACGACTGCTAATA
Stp.aureus               113 TGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATA

E.colirrsE               175 CCGCATAAC-------GTCGCAAGAC---------CAAAGAGGGGGACCTTCG-GGCCTCTTG
Cam.jejun5               176 CTCTATACTCCTGCTTAACACAAGTTGAGTAGG-GAAAG---------TTTTT-------CG
Stp.aureus               175 CCGGATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGT----CTT----GCTGTCA

E.colirrsE               221 CCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGA
Cam.jejun5               221 GTGTAGGATGAGACTATATAGTATCAGCTAGTTGGTAAGGTAATGGCTTACCAAGGCTATGA
Stp.aureus               229 CTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGA

E.colirrsE               283 TCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTA
Cam.jejun5               283 CGCTTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTA
Stp.aureus               291 TACGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTA
1659(COMPL)                                                             ACTCCTA
```

FIG. 89A

```
E.colirrsE      345 CGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTG
Cam.jejun5      345 CGGGAGGCAGCAGTAGGGAATATTGCGCAATGGGGGAAACCCTGACGCAGCAACGCCGCGTG
Stp.aureus      353 CGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTG
1659(COMPL)         CGGGAGGCAGCAG

E.colirrsE      407 TATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAA-GGGAGTAAAGTTAAT
Cam.jejun5      407 GAGGATGACACTTTTCGGAGCGTAAACTCCTTTTCTTAGGGAAG ----------AATT
Stp.aureus      415 AGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAAC

E.colirrsE      468 ACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCG
Cam.jejun5      455 C----------TGACGGTACCTAAGGAATAAGCACCGGCTAACTCCGTGCCAGCAGCCGCG
Stp.aureus      476 -TGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCG
```

FIG. 89B

```
E.coli rrsE      530 GTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTT
Cam.jejun5       506 GTAATACGGAGGGTGCAAGCGTTACTCGGAATCACTGGGCGTAAAGGGCGCGTAGGCGGATT
Stp.aureus       538 GTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTT

E.coli rrsE      592 GTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTT
Cam.jejun5       568 ATCAAGTCTCTTGTGAAATCTAATGGCTTAACCATTAAACTGCTTGGAAACTGATAGTCTA
Stp.aureus       600 TTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAACTT

E.coli rrsE      654 GAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGA
Cam.jejun5       630 GAGTGAGGAGAGGCAGATGGAATTGGTGGTGTAGGGGTAAAATCCGTAGATATCACCAAGA
Stp-aureus       662 GAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGA

E.coli rrsE      716 ATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGA
Cam.jejun5       692 ATACCCATTGCGAAGGCGATCTGCTGGAACTCAACTGACGCTAAGGCGCGAAAGCGTGGGGA
Stp.aureus       724 ACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGA

E.coli rrsE      778 GCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGC
Cam.jejun5       754 GCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTACACTAGTTGTTGGGGT
Stp.aureus       786 TCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGG
```

FIG. 89C

```
E.colirrsE   840 C-CTTGA-GGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGC
Cam.jejun5   816 G-CTAGT-CATCTCAGTAATGCAGCTAACGCATTAAGTGTACCGCCTGGGGAGTACGGTCGC
Stp.aureus   848 GT-TTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGC

E.colirrsE   900 AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATT
Cam.jejun5   876 AAGATTAAAACTCAAAGGAATAGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT
Stp.aureus   909 AAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT

E.colirrsE   962 CGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAAT
Cam.jejun5   938 CGAAGATACGCGAAGAACCTTACCTGGGCTTGATATCCTAAGAACCTTTTAGAGATAAGAGG
Stp.aureus   971 CGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCC

E.colirrsE  1024 GTG--CCTTCGGG--AA-CCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGA
Cam.jejun5  1000 GTGCTAGCTTGCTAGAA-CTTAGAGACAGGTGCTGCACGGCTGTCGTCAGCTCGTGTCGTGA
Stp.aureus  1033 TTCC-CCTTCGGG--GGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGA

SB-1                                  GCAACGAGCGCAACCC
E.colirrsE  1081 AATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGG-CC
Cam.jejun5  1061 GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCACGTATTTAGTTGCTAACGGTTCGG-CC
Stp.aureus  1092 GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCA-TTAAGT-T
```

FIG. 89D

```
SB-3 (SEQ ID NO:157)                                                    ATGACGTCAAGTCATC
SB-4 (SEQ ID NO:154)                                                    ATGACGTCAAGTCATC
E.colirrsE   1142 GGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATC
Cam.jejun5   1122 GAGCACTCTAAATAGACTGCCTTCG-TAAGGAGGAGGAAGGTGTGGACGACGTCAAGTCATC
Stp.aureus   1152 GGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATC

SB-3                ATGGCCCTTA
SB-4                ATGGCCCTTACGA
E.colirrsE   1204 ATGGCCCTTACGACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTC
Cam.jejun5   1183 ATGGCCCTTATGCCCAGGGCGACACACGTGCTACAATGGCATATAGAATGAGACGCAATACC
Stp.aureus   1214 ATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACC

E.colirrsE   1266 GCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTC
Cam.jejun5   1245 GCGAGGTGGAG-CAAATCTATAAAATATGTCCCAGTTCGGATTGTTCTCTGCAACTCGAGAG
Stp.aureus   1276 GCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTA

E.colirrsE   1328 CATGAAGTCGGAATCGCTAGTAATCGTGGATCAGA-ATGCCACGGTGAATACGTTCCCGGGC
Cam.jejun5   1306 CATGAAGCCGGAATCGCTAGTAATCGTAGATCAGCCATGCTACGGTGAATACGTTCCCGGGT
Stp.aureus   1338 CATGAAGCTGGAATCGCTAGTAATCGTAGATCAGC-ATGCTACGGTGAATACGTTCCCGGGT
1743(compl)                                                     CGGTGAATACGTTCCCGGGC
```

FIG. 89E

```
E.colirrsE   1389 CTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCT
Cam.jejun5   1368 CTTGTACTCACCGCCCGTCACACCATGGGAGTTGATTTCACTCGAAGCCGGAATACT--A-A
Stp.aureus   1399 ATTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCT
1743(compl)       CTTGTAC

E.colirrsE   1451 TCG-GGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGTAACAAGGTAACCG
Cam.jejun5   1427 AC---T-AGTTACCGTCCACAGTGGAATCAGCGACTGGGGTGAAGTCGTAACAAGGTAACCG
Stp.aureus   1461 TTTAGGAGCTAGCCGTCGAAGGTGGGACAAATGATTGGGGTGAAGTCGTAACAAGGTAGCCG

E.colirrsE   1512 TAGGGGAACCTGCGGTTGGATCACCTCCTTA---
Cam.jejun5   1485 TAGGAGAACCTGCGGTTGGATCACCTCCT-----
Stp.aureus   1523 TATCGGAAGGTGCGGCTGGATCACCTCCTTTCT-
```

FIG. 89F

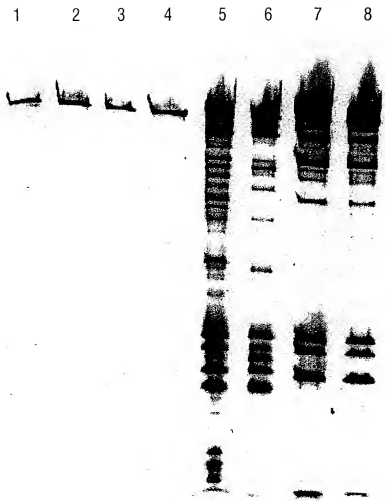


FIG. 90

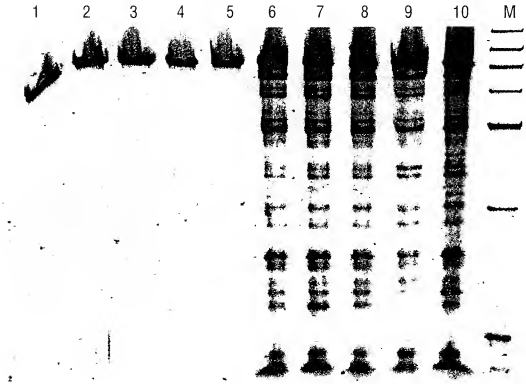


FIG. 91A

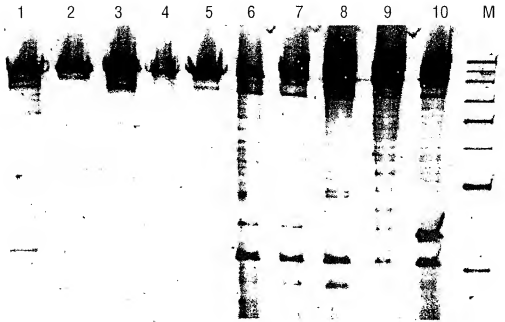


FIG. 91B

FIG. 92

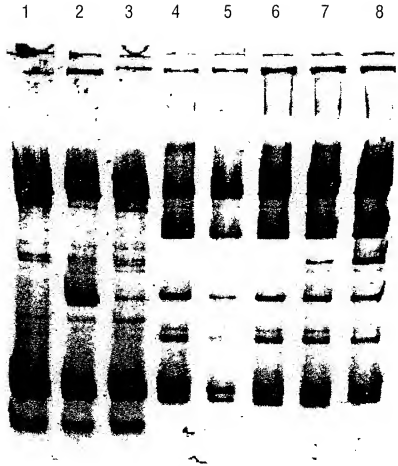


FIG. 93

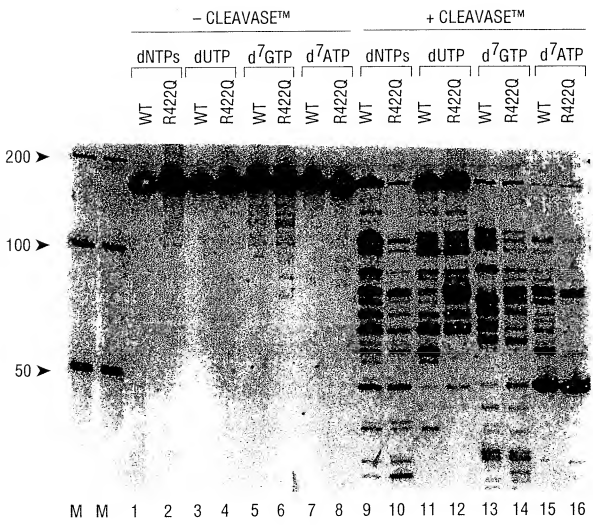

– CLEAVASE™
+ CLEAVASE™
dNTPs
dUTP
d7GTP
d7ATP
WT
R422Q
200
100
50
M M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

FIG. 94